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Gene expression in biological conditions

Technical field of the invention

The present invention relates to a method of predicting the prognosis of a biological condition in animal tissue, wherein the expression of genes is examined and correlated to standards. The invention further relates to the treatment of the biological condition and an assay for predicting the prognosis.

Background

The building of large databases containing human genome sequences is the basis for studies of gene expressions in various tissues during normal physiological and pathological conditions. Constantly (constitutively) expressed sequences as well as sequences whose expression is altered during disease processes are important for our understanding of cellular properties, and for the identification of candidate genes for future therapeutic intervention. As the number of known genes and ESTs build up in the databases, array-based simultaneous screening of thousands of genes is necessary to obtain a profile of transcriptional behaviour, and to identify key genes that either alone or in combination with other genes, control various aspects of cellular life. One cellular behaviour that has been a mystery for many years is the malignant behaviour of cancer cells. It is now known that for example defects in DNA repair can lead to cancer but the cancer-creating mechanism in heterozygous individuals is still largely unknown as is the malignant cell's ability to repeat cell cycles to avoid apoptosis to escape the immune system to invade and metastasize and to escape therapy. There are indications in these areas and excellent progress has been made, buth the myriad of genes interacting with each other in a highly complex multidimensional network is making the road to insight long and contorted.

Similar appearing tumors – morphologically, histochemically, microscopically – can be profoundly different. They can have different invasive and metastasizing properties, as well as respond differently to therapy. There is thus a need in the art for methods which distinguish tumors and tissues on factors different than those currently in clinical use.
The malignant transformation from normal tissue to cancer is believed to be a multistep process, in which tumorsuppressor genes, that normally repress cancer growth show reduced gene expression and in which other genes that encode tumor promoting proteins (oncogenes) show an increased expression level. Several tumor suppressor genes have been identified up till now, as e.g. p16, Rb, p53 (Nesrin Özören and Wafik S. El-Deiry, Introduction to cancer genes and growth control, In: DNA alterations in cancer, genetic and epigenetic changes, Eaton publishing, Melanie Ehrlich (ed) p. 1-43,

2000.; and references therein). They are usually identified by their lack of expression or their mutation in cancer tissue.

Other examinations have shown this downregulation of transcripts to be partly due to loss of genomic material (loss of heterozygosity), partly to methylation of promotorregions, and partly due to unknown factors (Nesrin Özören and Wafik S. El-Deiry, Introduction to cancer genes and growth control, In: DNA alterations in cancer, genetic and epigenetic changes, Eaton publishing, Melanie Ehrlich (ed) p. 1-43, 2000.; and references therein).

Several oncogenes are known, e.g. cyclinD1/PRAD1/BCL1, FGFs, c-MYC, BCL-2 all of which are genes that are amplified in cancer showing an increased level of transcript (Nesrin Özören and Wafik S. El-Deiry, Introduction to cancer genes and growth control, In: DNA alterations in cancer, genetic and epigenetic changes, Eaton publishing, Melanie Ehrlich (ed) p. 1-43, 2000.; and references therein). Many of these genes are related to cell growth and directs the tumor cells to uninhibited growth. Others may be related to tissue degradation as they e.g. encode enzymes that break down the surrounding connective tissue.

Bladder cancer is the fourth most common malignancy in males in the western countries (Pisani). The disease basically takes two different courses: one where patients have multiple recurrences of superficial tumors (Ta and T1), and one where the disease from the beginning is muscle invasive (T2+) and leads to metastasis. About 5-10% of patients with Ta tumors and 20-30% of the patients with T1 tumors will eventually develop a higher stage tumor (Wolf). Patients with superficial bladder tumors represent 75% of all bladder cancer patients and no clinical useful markers identifying patients with a poor prognosis exists at present.

The patients presenting isolated or concomitant Carcinoma in situ (CIS) lesions have a high risk of disease progression to a muscle invasive stage (Althausen). The CIS lesions may have a widespread manifestation in the bladder (field disease) and are believed to be the most common precursors of invasive carcinomas (Spruck, Rosin). The ability to predict which tumours are likely to recur or progress would have great impact on the clinical management of patients with superficial disease, as it would be possible to treat high-risk patients more aggressively (e.g. radical cystectomy or adjuvant therapy). This approach is currently not possible, as no clinical useful markers exist that identify these patients. Although many prognostic markers have been investigated, the most important prognostic factors are still disease stage, dysplasia grade and especially the presence of areas with CIS (Anderstrom, Cummings, Cheng). The gold standard for detection of CIS is urine cytology and histopathologic analysis of a set of selected site biopsies removed during routine

cytsocopy examinations; however these procedures are not sufficient sensitive. Implementing routine cytoscopy examinations with 5-ALA fluorescence imaging of the tumours and pre-cancerous lesions (CIS lesions and moderate dysplasia lesions) may increase the sensitivity of the procedure (Kriegmar), however, increased detection sensitivity is still necessary in order to offer better treatment regiments to the individual patients.

Summary of the invention

The present invention relates to prediction of prognosis of a biological condition, in particular to the prognosis of cancer such as bladder cancer. It is known that individuals suffering from cancer, although their tumors macroscopically and microscopically are identical, may have very different outcome. The present inventors have identified new predictor genes to classify macroscopically and microscopically identical tumors into two or more groups, wherein in each group has a separate risk profile of recurrence, invasive growth, metastasis etc. as compared to the other group(s). The present invention relates to genotyping of the tissue, and correlating the result to standard expression level(s) to predict the prognosis of the biological condition.

Accordingly, in one aspect the present invention relates to a method of predicting the prognosis of a biological condition in animal tissue,

comprising collecting a sample comprising cells from the tissue and/or expression products from the cells,

determining an expression level of at least one gene in said sample, said gene being selected from the group of genes consisting of gene No. 1 to gene No. 562,

correlating the expression level to at least one standard expression level to predict the prognosis of the biological condition in the animal tissue.

The genes No. 1 – gene No. 562 are found in table A described below herein.

Animal tissue may be tissue from any animal, preferably from a mammal, such as a horse, a cow, a dog, a cat, and more preferably the tissue is human tissue. The biological condition may be any condition exhibiting gene expression different from normal tissue. In particular the biological condition relates to a malignant or premalignant condition, such as a tumor or cancer, in particular bladder cancer. By the term "collecting a sample comprising cells" is meant the sample is provided in a manner, so that the expression level of the genes may be determined.

Furthermore, the invention relates to a method of determining the stage of a biological condition in animal tissue,

comprising collecting a sample comprising cells from the tissue,

determining an expression level of at least one gene in said sample, said gene being selected from the group of genes consisting of geneNo 1 to gene No. 562,

correlating the expression level of the assessed genes to at least one standard level of expression determining the stage of the condition.

The determination of the stage of the biological condition may be conducted prior to the method of predicting the method, or the stage of the biological condition may as such contain the information about the prognosis.

The methods above may be used for determining single gene expressions, however the invention also relates to a method of determining an expression pattern of a bladder cell sample, comprising:

collecting sample comprising bladder cells and/or expression products from bladder cells,

determining the expression level of at least one gene in the sample, said gene being selected from the group of genes consisting of gene No. 1 to gene No. 562, and obtaining an expression pattern of the bladder cell sample.

Further, the invention relates to a method of determining an expression pattern of a bladder cell sample independent of the proportion of submucosal, muscle, or connective tissue cells present, comprising:

determining the expression of one or more genes in a sample comprising cells, wherein the one or more genes exclude genes which are expressed in the submucosal, muscle, or connective tissue, whereby a pattern of expression is formed for the sample which is independent of the proportion of submucosal, muscle, or connective tissue cells in the sample.

The expression pattern may be used in a method according to this information, and accordingly, the invention also relates to a method of predicting the prognosis a biological condition in human bladder tissue comprising,

collecting a sample comprising cells from the tissue,

determining an expression pattern of the cells as defined in any of claims 43-54,

correlating the determined expression pattern to a standard pattern,

predicting the prognosis of the biological condition of said tissue

as well as a method for determining the stage of a biological condition in animal tissue, comprising

collecting a sample comprising cells from the tissue,

determining an expression pattern of the cells as defined above,

correlating the determined expression pattern to a standard pattern,

determining the stage of the biological condition is said tissue.

The invention further relates to a method for reducing cell tumorigenicity or malignancy of a cell, said method comprising

contacting a tumor cell with at least one peptide expressed by at least one gene selected from the group of genes consisting of gene Nos. 200-214, 233, 234, 235, 236, 244, 249, 251, 252, 255, 256, 259, 261, 262, 266, 268, 269, 273, 274, 275, 276, 277, 279, 280, 281, 282, 285, 286, 289, 293, 295, 296, 299, 301, 304, 306, 307, 308, 311, 312, 313, 314 , 320 , 322, 323, 325, 326, 327, 328 , 330, 331, 332, 333, 334, 338, 341, 342, 343, 345, 348, 349, 350, 351, 352, 353, 355, 357, 360, 361, 363, 366, 367, 370, 373, 374, 375, 376, 385, 386, 387, 389, 390, 392, 394, 398, 400, 401, 405, 406, 407, 408, 410, 411, 412, 414, 415, 416, 418, 424, 426, 428, 433, 434, 435, 436, 438, 439, 440, 441, 442, 443, 445, 446, 453, 460, 461, 463, 464, 465, 466, 467, 469, 470, 471, 472, 473, 475, 476, 477, 479, 480, 481, 482, 483, 485, 486, 487, 488, 490, 492, 494, 496, 497, 498 , 499, 503, 515, 516, 517, 521, 526, 527, 528, 530 ,532, 533, 537, 539, 540, 541, 542, 543, 545, 554, 557, 560 or

obtaining at least one gene selected from the group of genes consisting of gene Nos200-214, 233, 234, 235, 236, 244, 249, 251, 252, 255, 256, 259, 261, 262, 266, 268, 269, 273, 274, 275, 276, 277, 279, 280, 281, 282, 285, 286, 289, 293, 295, 296, 299, 301, 304, 306, 307, 308, 311, 312, 313, 314 , 320 , 322, 323, 325, 326, 327, 328 , 330, 331, 332, 333, 334,

338, 341, 342, 343, 345, 348, 349, 350, 351, 352, 353, 355, 357, 360, 361, 363, 366, 367, 370, 373, 374, 375, 376, 385, 386, 387, 389, 390, 392, 394, 398, 400, 401, 405, 406, 407, 408, 410, 411, 412, 414, 415, 416, 418, 424, 426, 428, 433, 434, 435, 436, 438, 439, 440, 441, 442, 443, 445, 446, 453, 460, 461, 463, 464, 465, 466, 467, 469, 470, 471, 472, 473, 475, 476, 477, 479, 480, 481, 482, 483, 485, 486, 487, 488, 490, 492, 494, 496, 497, 498 , 499, 503, 515, 516, 517, 521, 526, 527, 528, 530 ,532, 533, 537, 539, 540, 541, 542, 543, 545, 554, 557, 560, and introducing said at least one gene into the tumor cell in a manner allowing expression of said gene(s), or

obtaining at least one nucleotide probe capable of hybridising with at least one gene of a tumor cell, said at least one gene being selected from the group of genes consisting of gene Nos. 1-199, 215-232, 237, 238, 239, 240, 241, 242, 243, 245, 246, 247, 248, 250, 253, 254, 257, 258, 260, 263, 264, 265, 267, 270, 271, 272, 278, 283, 284, 287, 288, 290, 291, 292, 294, 297, 298, 300, 302, 303, 305, 309, 310, 315, 316, 317, 318, 319, 321, 324, 329, 335, 336, 337, 339, 340, 344, 346, 347, 354, 356, 358, 359, 362, 364, 365, 368, 369, 371, 372, 377, 378, 379, 380, 381, 382, 383, 384, 388, 391, 393, 395, 396, 397, 399, 402, 403, 404, 409, 413, 417, 419, 420, 421, 422, 423, 425, 427 ,429, 430, 431, 432, 437, 444, 447, 448, 449, 450, 451, 452, 454, 455 ,456, 457, 458, 459, 462, 468, 474, 478, 484, 489, 491, 493, 495, 500, 501, 502, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 518 , 519, 520, 522, 523, 524, 525, 529, 531, 534, 535, 536, 538, 544, 546, 547, 548, 549, 550, 551, 552, 553, 555, 556, 558, 559, 561, 562, and introducing said at least one nucleotide probe into the tumor cell in a manner allowing the probe to hybridise to the at least one gene, thereby inhibiting expression of said at least one gene.

In a further aspect the invention relates to a method for producing antibodies against an expression product of a cell from a biological tissue, said method comprising the steps of

obtaining expression product(s) from at least one gene said gene being expressed as defined above,

immunising a mammal with said expression product(s) obtaining antibodies against the expression product.

The antibodies produced may be used for producing a pharmaceutical composition. Further, the invention relates to a vaccine capable of eliciting an immune response against at least one expression product from at least one gene said gene being expressed as defined above.

The invention furthermore relates to the use of any of the methods discussed above for producing an assay for diagnosing a biological condition in animal tissue.

Also, the invention relates to the use of a peptide as defined above as an expression product and/or the use of a gene as defined above and/or the use of a probe as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

In yet a further aspect the invention relates to an assay for determining the presence or absence of a biological condition in animal tissue, comprising

at least one first marker capable of detecting an expression level of at least one gene selected from the group of genes consisting of gene No. 1 to gene No. 562,

In another aspect the invention relates to an assay for determining an expression pattern of a bladder cell, comprising at least a first marker and and/or a second marker, wherein the first marker is capable of detecting a gene from a first gene group as defined above, and the second marker is capable of detecting a gene from a second gene group as defined above.

Drawings

Description of figures:

Figure 1 Hierarchical cluster analysis of tumor samples based on 3,197 genes that show large variation across all tumor samples. Samples with progression are marked Prog.

Figure 2 Delineation of the 200 best marker genes. Genes that show higher levels of expression in the non-progression group are shown in the top and genes that show higher levels of expression in the progression group is shown in the bottom. Each column in the diagram represents a tumor sample and each row a gene. The 13 non-progressing samples are shown to the left and the 16 progressing samples are shown to the right in the diagram. The color saturation indicates differences in gene expression across the tumor samples; light color indicates up regulation compared the median expression and down regulation compared to the median expression of the gene is shown in dark color. Gene names of particular interesting genes are listed. Notable, non-group expression patterns were observed for two tumors (arrows). The tumor in the no progression group (150-6) showed a solid growth pattern, which is associated with a poor prognosis. No special tumor characteristics can help explain the gene expression pattern observed for the tumor in the progression group (825-3).

Figure 3. Cross-validation performance using from 1 to 200 genes.

Figure 4. Predicting progression in early stage bladder tumors. a, The 45-gene expression signature found to be optimal for progression prediction. Genes showing high expression in progressing samples are show in the top and genes showing high expression in the non-progressing samples are shown in the bottom. Genes are listed according to how many cross-validation loops included the genes. b, The 45-gene expression signature in the 19 tumor test-set. The samples are listed according to the correlation to the average non-progression signature from the training set samples. The read punctuated line separates samples with positive (left) and negative (right) correlation values. The white lines separates samples above and below the correlation cutoff values of 0.1 and –0.1. The sample legend indicates no-progression (N) samples and progression (P) samples.

Figure 5 Hierarchical cluster analysis of the metachronous tumor samples. Tight clustering tumors of different stage from the same patients are colored in grey.

Figure 6 Two-way hierarchical clustering and multidimensional scaling analysis of gene expression data from 40 bladder tumour biopsies. a, Tumour cluster dendrogram based on the 1767 gene-set. CIS annotations following the sample names indicate concomitant carcinoma in situ. Tumour recurrence rates are shown to the right of the dendrogram as + and ++ indicating moderate and high recurrence rates, respectively, while no sign indicates no or moderate recurrence. b, Tumour cluster dendrogram based on 88 cancer related genes. c, 2D plot of multidimensional scaling analysis of the 40 tumours based on the 1767 gene-set. The colour code identifies the tumour samples from the cluster dendrogram (Fig. 1a). d, Two-way cluster analysis diagram of the 1767 gene-set. Each row in the diagram represents a gene and each column a tumour sample. The colour saturation represents differences in gene expression across the tumour samples; lgiht color indicates higher expression of the gene compared to the median expression and lower expression of the gene compared to the median expression shown in dark color. The colour intensities indicate degrees of gene-regulation. The sidebars to the right of the diagram represent gene clusters a-j and normal 1-3 in the left side indicate the three normal biopsies and normal 4 indicates the pool of biopsies from 37 patients.

Figure 7 Enlarged view of the gene clusters a, c, f, and g. The dendrogram at the top is identical to Fig. 6a. a, Cluster of transcription factors and other nuclear associated genes. c, Cluster of genes involved in proliferation and cell cycle control. f, Gene expression pattern and corresponding area with squamous metaplasia in urothelial carcinoma. The light colour indicates genes up-regulated in samples 1178-1 and 875-1, the only two samples with squamous cell metaplasia. g, Cluster of genes involved in angiogenesis and matrix remodelling.

Figure 8. Hierarchical cluster analysis results
Here we show expanded views of clusters a-j as identified in the 1767 gene-cluster. The tumour cluster dendrogram and colour bars on top of the clusters represents the same tumour cluster as shown in the paper. The four samples to the left are normal biopsies (normal 1-3) and a pool of 37 normal biopsies (normal 4).

Figure 8a. Molecular classification of tumour samples using 80 predictive genes in each cross-validation loop. Each classification is based on the closeness to the mean in the three classes. Samples marked with * were not used to build the classifier. The scale indicates the distance from the samples to the classes in the classifier, measured in weighted squared Euclidean distance.

Figure 9 Number of classification errors vs. number of genes used in cross-validation loops.

Figure 10 Expression profiles of the 71 genes used in the final classifier model.The tumors shown are the 33 tumors used in the cross validation scheme. The Ta tumors are shown to the left, the T1 tumors in the middle, and the T2 tumors to the right.

Figure 11 Number of prediction errors vs. number of genes used in cross-validation loops.

Figure 12 The expression profiles of the 26 genes that constitute our final prediction model. The genes are listed according to the degree of correlation with the recurrence and non-recurrence groups. Genes with highest correlations are found in the top and the bottom of the list.

Figure 13 . Hierarchical cluster analysis of the gene expression in 41 TCC, 9 normal samples and 10 samples from cystectomy specimens with CIS lesions. a, Cluster dendrogram of all 41 TCC biopsies based on the expression of 5,491 genes. b, Cluster dendrogram of all superficial TCC biopsies based on the expression of 5,252 genes. c, Two-way cluster analysis diagram of the 41 TCC biopsies together with gene expressions in the normal and cystectomy samples (left columns). Each row represents a gene and each column represent a biopsy sample. Yellow indicates up-regulation compared to the median expression (black) of the gene and blue indicates down-regulation compared to the median expression. The colour saturation indicates degree of gene regulation. The sidebars to the right of the diagram represent gene-clusters 1-4; enlarged views of cluster 1 and 4 are shown to the right, with all gene symbols listed.

Figure 14 . Delineation of the 100 best markers that separate TCC without CIS from TCC with concomitant CIS. a, The 50 best up-regulated marker genes in TCC without CIS are shown in the top and the 50 best up-regulated marker genes in TCC with CIS are shown in the bottom. The gene symbols are listed to the right of the diagram. b, Expression profiles of the 100 marker genes in 9 normal biopsies (left column), 5 histologically normal samples adjacent to CIS lesions (middle column), and 5 biopsies with CIS lesions detected. (right column).

Figure 15 Cross validation performance using all samples

Figure 16 Expression profiles of the 16 genes in the CIS classifier. a, the expression of the 16 classifier genes in TCC with no surrounding CIS (left) and in TCC with surrounding CIS (right). The gene symbols of the classifier genes are listed together with the number of the times used in cross-validation loops. b, the expression of the 16 classifier genes in normal samples, in histologically normal samples adjacent to CIS lesions, and in biopsies with CIS lesions. The top dendrogram shows the sample clustering from hierarchical cluster analysis based on the 16 classifier genes. The genes appear in the same order as in 3a.

Figure 17 Cross validation performance using half of the samples

Figure 18 shows table B

Figure 19 shows table C

Figure 20 shows table D

Figure 21 shows table E

Figure 22 shows table F

Figure 23 shows table G

Figure 24 shows table H

Detailed description of the invention

As discussed above the present invention relates to the finding that it is possible to predict the prognosis of a biological condition by determining the expression level of one or more genes from a specified group of genes and comparing the expression level to at least one

standard for expression levels. The present inventors have identified 562 genes relevant for predicting the prognosis of a biological condition, in particular a cancer disease, such as bladder cancer.

The following table A shows the genes relevant in this context. Whenever a gene is cited herein with reference to a gene No. the numbering refers to the genes of Table A.

Table A

Gene #	GeneChip	Probeset	Unigene Build	Unigene	description	Classifier
1	HUGeneFL	AB000220_at	168	Hs.171921	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	stage
2	HUGeneFL	AF000231_at	168	Hs.75618	RAB11A, member RAS oncogene family	stage
3	HUGeneFL	D10922_s_at	168	Hs.99855	formyl peptide receptor-like 1	stage
4	HUGeneFL	D10925_at	168	Hs.301921	chemokine (C-C motif) receptor 1	stage
5	HUGeneFL	D11086_at	168	Hs.84	interleukin 2 receptor, gamma (severe combined immunodeficiency)	stage
6	HUGeneFL	D11151_at	168	Hs.211202	endothelin receptor type A	stage
7	HUGeneFL	D13435_at	168	Hs.426142	phosphatidylinositol glycan, class F	stage
8	HUGeneFL	D13666_s_at	168	Hs.136348	osteoblast specific factor 2 (fasciclin I-like)	stage
9	HUGeneFL	D14520_at	168	Hs.84728	Kruppel-like factor 5 (intestinal)	stage
10	HUGeneFL	D21878_at	168	Hs.169998	bone marrow stromal cell antigen 1	stage
11	HUGeneFL	D26443_at	168	Hs.371369	solute carrier family 1 (glial high affinity glutamate transporter), member 3	stage
12	HUGeneFL	D42046_at	168	Hs.194665	DNA2 DNA replication helicase 2-like (yeast)	stage
13	HUGeneFL	D45370_at	168	Hs.74120	adipose specific 2	stage
14	HUGeneFL	D49372_s_at	168	Hs.54460	chemokine (C-C motif) ligand 11	stage
15	HUGeneFL	D50495_at	168	Hs.224397	transcription elongation factor A (SII), 2	stage
16	HUGeneFL	D63135_at	168	Hs.27935	tweety homolog 2 (Drosophila)	stage
17	HUGeneFL	D64053_at	168	Hs.198288	protein tyrosine phosphatase, receptor type, R	stage
18	HUGeneFL	D83920_at	168	Hs.440898	ficolin (collagen/fibrinogen domain containing) 1	stage
19	HUGeneFL	D85131_s_at	168	Hs.433881	MYC-associated zinc finger protein (purine-binding transcription factor)	stage
20	HUGeneFL	D86062_s_at	168	Hs.413482	chromosome 21 open reading frame 33	stage
21	HUGeneFL	D86479_at	168	Hs.439463	AE binding protein 1	stage
22	HUGeneFL	D86957_at	168	Hs.307944	likely ortholog of mouse septin 8	stage
23	HUGeneFL	D86959_at	168	Hs.105751	Ste20-related serine/threonine kinase	stage
24	HUGeneFL	D86976_at	168	Hs.196914	minor histocompatibility antigen HA-1	stage
25	HUGeneFL	D87433_at	168	Hs.301989	stabilin 1	stage
26	HUGeneFL	D87443_at	168	Hs.409862	sorting nexin 19	stage
27	HUGeneFL	D87682_at	168	Hs.134792	KIAA0241 protein	stage
28	HUGeneFL	D89077_at	168	Hs.75367	Src-like-adaptor	stage
29	HUGeneFL	D89377_at	168	Hs.89404	msh homeo box homolog 2 (Drosophila)	stage
30	HUGeneFL	D90279_s_at	168	Hs.433695	collagen, type V, alpha 1	stage
31	HUGeneFL	HG1996-HT2044_at	168	—	—	stage
32	HUGeneFL	HG2090-HT2152_s_at	168	—	—	stage
33	HUGeneFL	HG2463-HT2559_at	168	—	—	stage
34	HUGeneFL	HG3044-HT3742_s_at	168	—	—	stage
35	HUGeneFL	HG3187-HT3366_s_at	168	—	—	stage
36	HUGeneFL	HG3342-HT3519_s_at	168	—	—	stage
37	HUGeneFL	HG371-HT26388_s_at	168	—	—	stage
38	HUGeneFL	HG4069-HT4339_s_at	168	—	—	stage
39	HUGeneFL	HG67-HT67_f_at	168	—	—	stage
40	HUGeneFL	HG907-	168	—	—	stage

		HT907_at				
41	HUGeneFL	J02871_s_at	168	Hs.436317	cytochrome P450, family 4, subfamily B, polypeptide 1	stage
42	HUGeneFL	J03040_at	168	Hs.111779	secreted protein, acidic, cysteine-rich (osteonectin)	stage
43	HUGeneFL	J03060_at	168	—	—	stage
44	HUGeneFL	J03068_at	168	—	—	stage
45	HUGeneFL	J03241_s_at	168	Hs.2025	transforming growth factor, beta 3	stage
46	HUGeneFL	J03278_at	168	Hs.307783	platelet-derived growth factor receptor, beta polypeptide	stage
47	HUGeneFL	J03909_at	168	—	—	stage
48	HUGeneFL	J03925_at	168	Hs.172631	integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)	stage
49	HUGeneFL	J04056_at	168	Hs.88778	carbonyl reductase 1	stage
50	HUGeneFL	J04058_at	168	Hs.169919	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)	stage
51	HUGeneFL	J04130_s_at	168	Hs.75703	chemokine (C-C motif) ligand 4	stage
52	HUGeneFL	J04152_rna1_s_at	168	—	—	stage
53	HUGeneFL	J04162_at	168	Hs.372679	Fc fragment of IgG, low affinity IIIa, receptor for (CD16)	stage
54	HUGeneFL	J04456_at	168	Hs.407909	lectin, galactoside-binding, soluble, 1 (galectin 1)	stage
55	HUGeneFL	J05032_at	168	Hs.32393	aspartyl-tRNA synthetase	stage
56	HUGeneFL	J05070_at	168	Hs.151738	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	stage
57	HUGeneFL	J05448_at	168	Hs.79402	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa	stage
58	HUGeneFL	K01396_at	168	Hs.297681	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	stage
59	HUGeneFL	K03430_at	168	—	—	stage
60	HUGeneFL	L06797_s_at	168	Hs.421986	chemokine (C-X-C motif) receptor 4	stage
61	HUGeneFL	L10343_at	168	Hs.112341	protease inhibitor 3, skin-derived (SKALP)	stage
62	HUGeneFL	L13391_at	168	Hs.78944	regulator of G-protein signalling 2, 24kDa	stage
63	HUGeneFL	L13698_at	168	Hs.65029	growth arrest-specific 1	stage
64	HUGeneFL	L13720_at	168	Hs.437710	growth arrest-specific 6	stage
65	HUGeneFL	L13923_at	168	Hs.750	fibrillin 1 (Marfan syndrome)	stage
66	HUGeneFL	L15409_at	168	Hs.421597	von Hippel-Lindau syndrome	stage
67	HUGeneFL	L17325_at	168	Hs.195825	RNA binding protein with multiple splicing	stage
68	HUGeneFL	L19872_at	168	Hs.170087	aryl hydrocarbon receptor	stage
69	HUGeneFL	L27476_at	168	Hs.75608	tight junction protein 2 (zona occludens 2)	stage
70	HUGeneFL	L33799_at	168	Hs.202097	procollagen C-endopeptidase enhancer	stage
71	HUGeneFL	L40388_at	168	Hs.30212	thyroid receptor interacting protein 15	stage
72	HUGeneFL	L40904_at	168	Hs.387667	peroxisome proliferative activated receptor, gamma	stage
73	HUGeneFL	L41919_rna1_at	168	—	—	stage
74	HUGeneFL	M11433_at	168	Hs.101850	retinol binding protein 1, cellular	stage
75	HUGeneFL	M11718_at	168	Hs.283393	collagen, type V, alpha 2	stage
76	HUGeneFL	M12125_at	168	Hs.300772	tropomyosin 2 (beta)	stage
77	HUGeneFL	M14218_at	168	Hs.442047	argininosuccinate lyase	stage
78	HUGeneFL	M15395_at	168	Hs.375957	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	stage
79	HUGeneFL	M16591_s_at	168	Hs.89555	hemopoietic cell kinase	stage
80	HUGeneFL	M17219_at	168	Hs.203862	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	stage
81	HUGeneFL	M20530_at	168	—	—	stage
82	HUGeneFL	M23178_s_at	168	Hs.73817	chemokine (C-C motif) ligand 3	stage
83	HUGeneFL	M28130_rna1_s_at	168	—	—	stage
84	HUGeneFL	M29550_at	168	Hs.187543	protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)	stage
85	HUGeneFL	M31165_at	168	Hs.407546	tumor necrosis factor, alpha-induced protein 6	stage
86	HUGeneFL	M32011_at	168	Hs.949	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	stage
87	HUGeneFL	M33195_at	168	Hs.433300	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	stage
88	HUGeneFL	M37033_at	168	Hs.443057	CD53 antigen	stage
89	HUGeneFL	M37766_at	168	Hs.901	CD48 antigen (B-cell membrane protein)	stage

90	HUGeneFL	M55998_s_at	168	Hs.172928	collagen, type I, alpha 1	stage
91	HUGeneFL	M57731_s_at	168	Hs.75765	chemokine (C-X-C motif) ligand 2	stage
92	HUGeneFL	M62840_at	168	Hs.82542	acyloxyacyl hydrolase (neutrophil)	stage
93	HUGeneFL	M63262_at	168	—	—	stage
94	HUGeneFL	M68840_at	168	Hs.183109	monoamine oxidase A	stage
95	HUGeneFL	M69203_s_at	168	Hs.75703	chemokine (C-C motif) ligand 4	stage
96	HUGeneFL	M72885_rna1_s_at	168	—	—	stage
97	HUGeneFL	M77349_at	168	Hs.421496	transforming growth factor, beta-induced, 68kDa	stage
98	HUGeneFL	M82882_at	168	Hs.124030	E74-like factor 1 (ets domain transcription factor)	stage
99	HUGeneFL	M83822_at	168	Hs.209846	LPS-responsive vesicle trafficking, beach and anchor containing	stage
100	HUGeneFL	M92934_at	168	Hs.410037	connective tissue growth factor	stage
101	HUGeneFL	M95178_at	168	Hs.119000	actinin, alpha 1	stage
102	HUGeneFL	S69115_at	168	Hs.10306	natural killer cell group 7 sequence	stage
103	HUGeneFL	S77393_at	168	Hs.145754	Kruppel-like factor 3 (basic)	stage
104	HUGeneFL	S78187_at	168	Hs.153752	cell division cycle 25B	stage
105	HUGeneFL	U01833_at	168	Hs.81469	nucleotide binding protein 1 (MinD homolog, E. coli)	stage
106	HUGeneFL	U07231_at	168	Hs.309763	G-rich RNA sequence binding factor 1	stage
107	HUGeneFL	U09278_at	168	Hs.436852	fibroblast activation protein, alpha	stage
108	HUGeneFL	U09937_rna1_s_at	168	—	—	stage
109	HUGeneFL	U10550_at	168	Hs.79022	GTP binding protein overexpressed in skeletal muscle	stage
110	HUGeneFL	U12424_s_at	168	Hs.108646	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	stage
111	HUGeneFL	U16306_at	168	Hs.434488	chondroitin sulfate proteoglycan 2 (versican)	stage
112	HUGeneFL	U20158_at	168	Hs.2488	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)	stage
113	HUGeneFL	U20536_s_at	168	Hs.3280	caspase 6, apoptosis-related cysteine protease	stage
114	HUGeneFL	U24266_at	168	Hs.77448	aldehyde dehydrogenase 4 family, member A1	stage
115	HUGeneFL	U28249_at	168	Hs.301350	FXYD domain containing ion transport regulator 3	stage
116	HUGeneFL	U28488_s_at	168	Hs.155935	complement component 3a receptor 1	stage
117	HUGeneFL	U29680_at	168	Hs.227817	BCL2-related protein A1	stage
118	HUGeneFL	U37143_at	168	Hs.152096	cytochrome P450, family 2, subfamily J, polypeptide 2	stage
119	HUGeneFL	U38864_at	168	Hs.108139	zinc finger protein 212	stage
120	HUGeneFL	U39840_at	168	Hs.163484	forkhead box A1	stage
121	HUGeneFL	U41315_rna1_s_at	168	—	—	stage
122	HUGeneFL	U44111_at	168	Hs.42151	histamine N-methyltransferase	stage
123	HUGeneFL	U47414_at	168	Hs.13291	cyclin G2	stage
124	HUGeneFL	U49352_at	168	Hs.414754	2,4-dienoyl CoA reductase 1, mitochondrial	stage
125	HUGeneFL	U50708_at	168	Hs.1265	branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)	stage
126	HUGeneFL	U52101_at	168	Hs.9999	epithelial membrane protein 3	stage
127	HUGeneFL	U59914_at	168	Hs.153863	MAD, mothers against decapentaplegic homolog 6 (Drosophila)	stage
128	HUGeneFL	U60205_at	168	Hs.393239	sterol-C4-methyl oxidase-like	stage
129	HUGeneFL	U61981_at	168	Hs.42674	mutS homolog 3 (E. coli)	stage
130	HUGeneFL	U64520_at	168	Hs.66708	vesicle-associated membrane protein 3 (cellubrevin)	stage
131	HUGeneFL	U65093_at	168	Hs.82071	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	stage
132	HUGeneFL	U66619_at	168	Hs.444445	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	stage
133	HUGeneFL	U68019_at	168	Hs.288261	MAD, mothers against decapentaplegic homolog 3 (Drosophila)	stage
134	HUGeneFL	U68385_at	168	Hs.380923	likely ortholog of mouse myeloid ecotropic viral integration site-related gene 2	stage
135	HUGeneFL	U68485_at	168	Hs.193163	bridging integrator 1	stage
136	HUGeneFL	U74324_at	168	Hs.90875	RAB interacting factor	stage
137	HUGeneFL	U77970_at	168	Hs.321164	neuronal PAS domain protein 2	stage
138	HUGeneFL	U83303_cds2_at	168	Hs.164021	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	stage
139	HUGeneFL	U88871_at	168	Hs.79993	peroxisomal biogenesis factor 7	stage

140	HUGeneFL	U90549_at	168	Hs.236774	high mobility group nucleosomal binding domain 4	stage
141	HUGeneFL	U90716_at	168	Hs.79187	coxsackie virus and adenovirus receptor	stage
142	HUGeneFL	V00594_at	168	Hs.118786	metallothionein 2A	stage
143	HUGeneFL	V00594_s_at	168	Hs.118786	metallothionein 2A	stage
144	HUGeneFL	X02761_s_at	168	Hs.418138	fibronectin 1	stage
145	HUGeneFL	X04011_at	168	Hs.88974	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	stage
146	HUGeneFL	X04085_rna1_at	168	—	—	stage
147	HUGeneFL	X07438_s_at	168	—	—	stage
148	HUGeneFL	X07743_at	168	Hs.77436	pleckstrin	stage
149	HUGeneFL	X13334_at	168	Hs.75627	CD14 antigen	stage
150	HUGeneFL	X14046_at	168	Hs.153053	CD37 antigen	stage
151	HUGeneFL	X14813_at	168	Hs.166160	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	stage
152	HUGeneFL	X15880_at	168	Hs.415997	collagen, type VI, alpha 1	stage
153	HUGeneFL	X15882_at	168	Hs.420269	collagen, type VI, alpha 2	stage
154	HUGeneFL	X51408_at	168	Hs.380138	chimerin (chimaerin) 1	stage
155	HUGeneFL	X53800_s_at	168	Hs.89690	chemokine (C-X-C motif) ligand 3	stage
156	HUGeneFL	X54489_rna1_at	168	—	—	stage
157	HUGeneFL	X57351_s_at	168	Hs.174195	interferon induced transmembrane protein 2 (1-8D)	stage
158	HUGeneFL	X57579_s_at	168	—	—	stage
159	HUGeneFL	X58072_at	168	Hs.169946	GATA binding protein 3	stage
160	HUGeneFL	X62048_at	168	Hs.249441	WEE1 homolog (S. pombe)	stage
161	HUGeneFL	X64072_s_at	168	Hs.375957	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	stage
162	HUGeneFL	X65614_at	168	Hs.2962	S100 calcium binding protein P	stage
163	HUGeneFL	X66945_at	168	Hs.748	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	stage
164	HUGeneFL	X67491_f_at	168	Hs.355697	glutamate dehydrogenase 1	stage
165	HUGeneFL	X68194_at	168	Hs.80919	synaptophysin-like protein	stage
166	HUGeneFL	X73882_at	168	Hs.254605	microtubule-associated protein 7	stage
167	HUGeneFL	X78520_at	168	Hs.372528	chloride channel 3	stage
168	HUGeneFL	X78549_at	168	Hs.51133	PTK6 protein tyrosine kinase 6	stage
169	HUGeneFL	X78565_at	168	Hs.98998	tenascin C (hexabrachion)	stage
170	HUGeneFL	X78669_at	168	Hs.79088	reticulocalbin 2, EF-hand calcium binding domain	stage
171	HUGeneFL	X83618_at	168	Hs.59889	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	stage
172	HUGeneFL	X84908_at	168	Hs.78060	phosphorylase kinase, beta	stage
173	HUGeneFL	X90908_at	168	Hs.147391	fatty acid binding protein 6, ileal (gastrotropin)	stage
174	HUGeneFL	X91504_at	168	Hs.389277	ADP-ribosylation factor related protein 1	stage
175	HUGeneFL	X95632_s_at	168	Hs.387906	abl-interactor 2	stage
176	HUGeneFL	X97267_rna1_s_at	168	—	—	stage
177	HUGeneFL	Y00705_at	168	Hs.407856	serine protease inhibitor, Kazal type 1	stage
178	HUGeneFL	Y00787_s_at	168	Hs.624	interleukin 8	stage
179	HUGeneFL	Y00815_at	168	Hs.75216	protein tyrosine phosphatase, receptor type, F	stage
180	HUGeneFL	Y08374_rna1_at	168	—	—	stage
181	HUGeneFL	Z12173_at	168	Hs.334534	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	stage
182	HUGeneFL	Z19554_s_at	168	Hs.435800	vimentin	stage
183	HUGeneFL	Z26491_s_at	168	Hs.240013	catechol-O-methyltransferase	stage
184	HUGeneFL	Z29331_at	168	Hs.372758	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	stage
185	HUGeneFL	Z35491_at	168	Hs.377484	BCL2-associated athanogene	stage
186	HUGeneFL	Z48199_at	168	Hs.82109	syndecan 1	stage
187	HUGeneFL	Z48605_at	168	Hs.421825	inorganic pyrophosphatase 2	stage
188	HUGeneFL	Z74615_at	168	Hs.172928	collagen, type I, alpha 1	stage
189	HUGeneFL	D87437_at	168	Hs.43660	chromosome 1 open reading frame 16	recurrence
190	HUGeneFL	L49169_at	168	Hs.75678	FBJ murine osteosarcoma viral oncogene homolog B	recurrence
191	HUGeneFL	AF006041_at	168	Hs.336916	death-associated protein 6	recurrence
192	HUGeneFL	D83780_at	168	Hs.437991	KIAA0196 gene product	recurrence
193	HUGeneFL	D64154_at	168	Hs.90107	adhesion regulating molecule 1	recur-

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194	HUGeneFL	D21337_at	168	Hs.408	collagen, type IV, alpha 6	recur-rence
195	HUGeneFL	M16938_s_at	168	Hs.820	homeo box C6	recur-rence
196	HUGeneFL	D87258_at	168	Hs.75111	protease, serine, 11 (IGF binding)	recur-rence
197	HUGeneFL	U58516_at	168	Hs.3745	milk fat globule-EGF factor 8 protein	recur-rence
198	HUGeneFL	U45973_at	168	Hs.178347	skeletal muscle and kidney enriched inositol phosphatase	recur-rence
199	HUGeneFL	U62015_at	168	Hs.8867	cysteine-rich, angiogenic inducer, 61	recur-rence
200	HUGeneFL	U94855_at	168	Hs.381255	eukaryotic translation initiation factor 3, sub-unit 5 epsilon, 47kDa	recur-rence
201	HUGeneFL	L34155_at	168	Hs.83450	laminin, alpha 3	recur-rence
202	HUGeneFL	U70439_s_at	168	Hs.84264	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	recur-rence
203	HUGeneFL	U66702_at	168	Hs.74624	protein tyrosine phosphatase, receptor type, N polypeptide 2	recur-rence
204	HUGeneFL	HG511-HT511_at	168	—	—	recur-rence
205	HUGeneFL	HG3076-HT3238_s_at	168	—	—	recur-rence
206	HUGeneFL	M98528_at	168	Hs.79404	DNA segment on chromosome 4 (unique) 234 expressed sequence	recur-rence
207	HUGeneFL	M63175_at	168	Hs.295137	autocrine motility factor receptor	recur-rence
208	HUGeneFL	D49387_at	168	Hs.294584	leukotriene B4 12-hydroxydehydrogenase	recur-rence
209	HUGeneFL	HG1879-HT1919_at	168	—	—	recur-rence
210	HUGeneFL	Z23064_at	168	Hs.380118	RNA binding motif protein, X chromosome	recur-rence
211	HUGeneFL	X63469_at	168	Hs.77100	general transcription factor IIE, polypeptide 2, beta 34kDa	recur-rence
212	HUGeneFL	L38928_at	168	Hs.118131	5,10-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)	recur-rence
213	HUGeneFL	U21858_at	168	Hs.60679	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa	recur-rence
214	HUGeneFL	M64572_at	168	Hs.405666	protein tyrosine phosphatase, non-receptor type 3	recur-rence
215	HUGeneFL	D83657_at	168	Hs.19413	S100 calcium binding protein A12 (calgranulin C)	SCC
216	HUGeneFL	HG3945-HT4215_at	168	—	—	SCC
217	HUGeneFL	J00124_at	168	—	—	SCC
218	HUGeneFL	L05187_at	168	—	—	SCC
219	HUGeneFL	L42583_f_at	168	Hs.367762	keratin 6A	SCC
220	HUGeneFL	L42601_f_at	168	Hs.367762	keratin 6A	SCC
221	HUGeneFL	L42611_f_at	168	Hs.446417	keratin 6E	SCC
222	HUGeneFL	M19888_at	168	Hs.1076	small proline-rich protein 1B (cornifin)	SCC
223	HUGeneFL	M20030_f_at	168	Hs.505352	Human small proline rich protein (sprII) mRNA, clone 930.	SCC
224	HUGeneFL	M21005_at	168	—	—	SCC
225	HUGeneFL	M21302_at	168	Hs.505327	Human small proline rich protein (sprII) mRNA, clone 174N.	SCC
226	HUGeneFL	M21539_at	168	Hs.2421	small proline-rich protein 2C	SCC
227	HUGeneFL	M86757_s_at	168	Hs.112408	S100 calcium binding protein A7 (psoriasin 1)	SCC
228	HUGeneFL	S72493_s_at	168	Hs.432448	keratin 16 (focal non-epidermolytic palmoplan-tar keratoderma)	SCC
229	HUGeneFL	U70981_at	168	Hs.336046	interleukin 13 receptor, alpha 2	SCC
230	HUGeneFL	V01516_f_at	168	Hs.367762	keratin 6A	SCC
231	HUGeneFL	X53065_f_at	168	—	—	SCC
232	HUGeneFL	X57766_at	168	Hs.143751	matrix metalloproteinase 11 (stromelysin 3)	SCC
233	EOS Hu03	400773	133	-	NM_003105*:Homo sapiens sortilin-related receptor, L(DLR class) A repeats-containing (SORL1), mRNA.	progres-sion
234	EOS Hu03	400843	133	-	NM_003105*:Homo sapiens sortilin-related receptor, L(DLR class) A repeats-containing (SORL1), mRNA.	progres-sion
235	EOS Hu03	400844	133	-	NM_003105*:Homo sapiens sortilin-related	pro-

					receptor, L(DLR class) A repeats-containing (SORL1), mRNA.	gres-sion
236	EOS Hu03	400846	133	-	sortilin-related receptor, L(DLR class) A repeats-containing (SORL1)	progres-sion
237	EOS Hu03	402328	133	-	Target Exon	progres-sion
238	EOS Hu03	402384	133	-	NM_007181*:Homo sapiens mitogen-activated protein kinase kinase kinase kinase 1 (MAP4K1), mRNA.	progres-sion
239	EOS Hu03	404208	133	-	C6001282:gi|4504223|ref|NP_000172.1| glucuronidase, beta [Homo sapiens] gi|114963|sp|P082	progres-sion
240	EOS Hu03	404606	133	-	Target Exon	progres-sion
241	EOS Hu03	404826	133	-	Target Exon	progres-sion
242	EOS Hu03	404875	133	-	NM_022819*:Homo sapiens phospholipase A2, group IIF (PLA2G2F), mRNA. VERSION NM_020245.2 GI	progres-sion
243	EOS Hu03	404913	133	-	NM_024408*:Homo sapiens Notch (Drosophila) homolog 2 (NOTCH2), mRNA. VERSION NM_024410.1 GI	progres-sion
244	EOS Hu03	404977	133	-	Insulin-like growth factor 2 (somatomedin A) (IGF2)	progres-sion
245	EOS Hu03	405036	133	-	NM_021628*:Homo sapiens arachidonate lipoxygenase 3 (ALOXE3), mRNA. VERSION NM_020229.1 GI	progres-sion
246	EOS Hu03	405371	133	-	NM_005569*:Homo sapiens LIM domain kinase 2 (LIMK2), transcript variant 2a, mRNA.	progres-sion
247	EOS Hu03	405667	133	-	Target Exon	progres-sion
248	EOS Hu03	406002	133	-	Target Exon	progres-sion
249	EOS Hu03	407955	133	Hs.9343	ESTs	progres-sion
250	EOS Hu03	408049	133	Hs.345588	desmoplakin (DPI, DPII)	progres-sion
251	EOS Hu03	408288	133	Hs.16886	gb:zl73d06.r1 Stratagene colon (937204) Homo sapiens cDNA clone 5', mRNA sequence	progres-sion
252	EOS Hu03	409513	133	Hs.54642	methionine adenosyltransferase II, beta	progres-sion
253	EOS Hu03	409556	133	Hs.54941	phosphorylase kinase, alpha 2 (liver)	progres-sion
254	EOS Hu03	409586	133	Hs.55044	DKFZP586H2123 protein	progres-sion
255	EOS Hu03	409632	133	Hs.55279	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	progres-sion
256	EOS Hu03	410047	133	Hs.379753	zinc finger protein 36 (KOX 18)	progres-sion
257	EOS Hu03	411817	133	Hs.72241	mitogen-activated protein kinase kinase 2	progres-sion
258	EOS Hu03	412649	133	Hs.74369	integrin, alpha 7	progres-sion
259	EOS Hu03	412841	133	Hs.101395	hypothetical protein MGC11352	progres-

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260	EOS Hu03	413564	133		gb:601146990F1 NIH_MGC_19 Homo sapiens cDNA clone 5', mRNA sequence	progression
261	EOS Hu03	413786	133	Hs.13500	ESTs	progression
262	EOS Hu03	413840	133	Hs.356228	RNA binding motif protein, X chromosome	progression
263	EOS Hu03	413929	133	Hs.75617	collagen, type IV, alpha 2	progression
264	EOS Hu03	414223	133	Hs.238246	hypothetical protein FLJ22479	progression
265	EOS Hu03	414732	133	Hs.77152	minichromosome maintenance deficient (S. cerevisiae) 7	progression
266	EOS Hu03	414762	133	Hs.77257	KIAA0068 protein	progression
267	EOS Hu03	414840	133	Hs.23823	hairy/enhancer-of-split related with YRPW motif-like	progression
268	EOS Hu03	414843	133	Hs.77492	heterogeneous nuclear ribonucleoprotein A0	progression
269	EOS Hu03	414895	133	Hs.116278	Homo sapiens cDNA FLJ13571 fis, clone PLACE1008405	progression
270	EOS Hu03	414907	133	Hs.77597	polo (Drosophia)-like kinase	progression
271	EOS Hu03	414918	133	Hs.72222	hypothetical protein FLJ13459	progression
272	EOS Hu03	415200	133	Hs.78202	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	progression
273	EOS Hu03	416640	133	Hs.79404	neuron-specific protein	progression
274	EOS Hu03	416815	133	Hs.80120	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)	progression
275	EOS Hu03	416977	133	Hs.406103	hypothetical protein FKSG44	progression
276	EOS Hu03	417615	133	Hs.82314	hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	progression
277	EOS Hu03	417839	133	Hs.82712	fragile X mental retardation, autosomal homolog 1	progression
278	EOS Hu03	417900	133	Hs.82906	CDC20 (cell division cycle 20, S. cerevisiae, homolog)	progression
279	EOS Hu03	417924	133	Hs.82932	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	progression
280	EOS Hu03	418127	133	Hs.83532	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	progression
281	EOS Hu03	418321	133	Hs.84087	KIAA0143 protein	progression
282	EOS Hu03	418504	133	Hs.85335	Homo sapiens mRNA; cDNA DKFZp564D1462 (from clone DKFZp564D1462)	progression
283	EOS Hu03	418629	133	Hs.86859	growth factor receptor-bound protein 7	progres-

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284	EOS Hu03	419602	133	Hs.91521	hypothetical protein	progression
285	EOS Hu03	419847	133	Hs.184544	Homo sapiens, clone IMAGE:3355383, mRNA, partial cds	progression
286	EOS Hu03	420079	133	Hs.94896	PTD011 protein	progression
287	EOS Hu03	420116	133	Hs.95231	FH1/FH2 domain-containing protein	progression
288	EOS Hu03	420307	133	Hs.66219	ESTs	progression
289	EOS Hu03	420613	133	Hs.406637	ESTs, Weakly similar to A47582 B-cell growth factor precursor [H.sapiens]	progression
290	EOS Hu03	420732	133	Hs.367762	ESTs	progression
291	EOS Hu03	421026	133	Hs.101067	GCN5 (general control of amino-acid synthesis, yeast, homolog)-like 2	progression
292	EOS Hu03	421075	133	Hs.101474	KIAA0807 protein	progression
293	EOS Hu03	421101	133	Hs.101840	major histocompatibility complex, class I-like sequence	progression
294	EOS Hu03	421186	133	Hs.270563	ESTs, Moderately similar to T12512 hypothetical protein DKFZp434G232.1 [H.sapiens]	progression
295	EOS Hu03	421311	133	Hs.283609	hypothetical protein PRO2032	progression
296	EOS Hu03	421475	133	Hs.104640	HIV-1 inducer of short transcripts binding protein; lymphoma related factor	progression
297	EOS Hu03	421505	133	Hs.285641	KIAA1111 protein	progression
298	EOS Hu03	421595	133	Hs.301685	KIAA0620 protein	progression
299	EOS Hu03	421628	133	Hs.106210	hypothetical protein FLJ10813	progression
300	EOS Hu03	421649	133	Hs.106415	peroxisome proliferative activated receptor, delta	progression
301	EOS Hu03	421733	133	Hs.1420	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	progression
302	EOS Hu03	421782	133	Hs.108258	actin binding protein; macrophin (microfilament and actin filament cross-linker protein)	progression
303	EOS Hu03	421989	133	Hs.110457	Wolf-Hirschhorn syndrome candidate 1	progression
304	EOS Hu03	422043	133	Hs.110953	retinoic acid induced 1	progression
305	EOS Hu03	422068	133	Hs.104520	Homo sapiens cDNA FLJ13694 fis, clone PLACE2000115	progression
306	EOS Hu03	422506	133	Hs.300741	sorcin	progression
307	EOS Hu03	422913	133	Hs.121599	CGI-18 protein	progression

308	EOS Hu03	422929	133	Hs.94011	ESTs, Weakly similar to MGB4_HUMAN MELANOMA-ASSOCIATED ANTIGEN B4 [H.sapiens]	progression
309	EOS Hu03	422959	133	Hs.349256	paired immunoglobulin-like receptor beta	progression
310	EOS Hu03	423138	133	-	gb:EST385571 MAGE resequences, MAGM Homo sapiens cDNA, mRNA sequence	progression
311	EOS Hu03	423185	133	Hs.380062	ornithine decarboxylase antizyme 1	progression
312	EOS Hu03	423599	133	Hs.31731	peroxiredoxin 5	progression
313	EOS Hu03	423810	133	Hs.132955	BCL2/adenovirus E1B 19kD-interacting protein 3-like	progression
314	EOS Hu03	423960	133	Hs.136309	SH3-containing protein SH3GLB1	progression
315	EOS Hu03	424244	133	Hs.143601	hypothetical protein hCLA-iso	progression
316	EOS Hu03	424415	133	Hs.146580	enolase 2, (gamma, neuronal)	progression
317	EOS Hu03	424909	133	Hs.153752	cell division cycle 25B	progression
318	EOS Hu03	424959	133	Hs.153937	activated p21cdc42Hs kinase	progression
319	EOS Hu03	425093	133	Hs.154525	KIAA1076 protein	progression
320	EOS Hu03	425097	133	Hs.154545	PDZ domain containing guanine nucleotide exchange factor(GEF)1	progression
321	EOS Hu03	425205	133	Hs.155106	receptor (calcitonin) activity modifying protein 2	progression
322	EOS Hu03	425221	133	Hs.155188	TATA box binding protein (TBP)-associated factor, RNA polymerase II, F, 55kD	progression
323	EOS Hu03	425243	133	Hs.155291	KIAA0005 gene product	progression
324	EOS Hu03	425380	133	Hs.32148	AD-015 protein	progression
325	EOS Hu03	426028	133	Hs.172028	a disintegrin and metalloproteinase domain 10 (ADAM10)	progression
326	EOS Hu03	426125	133	Hs.166994	FAT tumor suppressor (Drosophila) homolog	progression
327	EOS Hu03	426177	133	Hs.167700	Homo sapiens cDNA FLJ10174 fis, clone HEMBA1003959	progression
328	EOS Hu03	426252	133	Hs.28917	ESTs	progression
329	EOS Hu03	426468	133	Hs.117558	ESTs	progression
330	EOS Hu03	426469	133	Hs.363039	methylmalonate-semialdehyde dehydrogenase	progression
331	EOS Hu03	426508	133	Hs.170171	glutamate-ammonia ligase (glutamine synthase)	progression
332	EOS Hu03	426682	133	Hs.2056	UDP glycosyltransferase 1 family, polypeptide	pro-

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333	EOS Hu03	426799	133	Hs.303154	popeye protein 3	progres-sion
334	EOS Hu03	426982	133	Hs.173091	ubiquitin-like 3	progres-sion
335	EOS Hu03	427239	133	Hs.356512	ubiquitin carrier protein	progres-sion
336	EOS Hu03	427351	133	Hs.123253	hypothetical protein FLJ22009	progres-sion
337	EOS Hu03	427681	133	Hs.284232	tumor necrosis factor receptor superfamily, member 12 (translocating chain-association membrane protein)	progres-sion
338	EOS Hu03	427722	133	Hs.180479	hypothetical protein FLJ20116	progres-sion
339	EOS Hu03	427747	133	Hs.180655	serine/threonine kinase 12	progres-sion
340	EOS Hu03	427999	133	Hs.181369	ubiquitin fusion degradation 1-like	progres-sion
341	EOS Hu03	428115	133	Hs.300855	KIAA0977 protein	progres-sion
342	EOS Hu03	428284	133	Hs.183435	NM_004545:Homo sapiens NADH dehydro-genase (ubiquinone) 1 beta subcomplex, 1 (7kD, MNLL) (NDUFB1), mRNA.	progres-sion
343	EOS Hu03	428318	133	Hs.356190	ubiquitin B	progres-sion
344	EOS Hu03	428712	133	Hs.190452	KIAA0365 gene product	progres-sion
345	EOS Hu03	428901	133	Hs.146668	KIAA1253 protein	progres-sion
346	EOS Hu03	429124	133	Hs.196914	minor histocompatibility antigen HA-1	progres-sion
347	EOS Hu03	429187	133	Hs.163872	ESTs, Weakly similar to S65657 alpha-1C-adrenergic receptor splice form 2 [H.sapiens]	progres-sion
348	EOS Hu03	429311	133	Hs.198998	conserved helix-loop-helix ubiquitous kinase	progres-sion
349	EOS Hu03	429561	133	Hs.250646	baculoviral IAP repeat-containing 6	progres-sion
350	EOS Hu03	429802	133	Hs.5367	ESTs, Weakly similar to I38022 hypothetical protein [H.sapiens]	progres-sion
351	EOS Hu03	429953	133	Hs.226581	COX15 (yeast) homolog, cytochrome c oxi-dase assembly protein	progres-sion
352	EOS Hu03	430604	133	Hs.247309	succinate-CoA ligase, GDP-forming, beta subunit	progres-sion
353	EOS Hu03	430677	133	Hs.359784	desmoglein 2	progres-sion
354	EOS Hu03	430746	133	Hs.406256	ESTs	progres-sion
355	EOS Hu03	431604	133	Hs.264190	vacuolar protein sorting 35 (yeast homolog)	progres-sion
356	EOS Hu03	431842	133	Hs.271473	epithelial protein up-regulated in carcinoma, membrane associated protein 17	progres-

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357	EOS Hu03	431857	133	Hs.271742	ADP-ribosyltransferase (NAD; poly (ADP-ribose) polymerase)-like 3	progression
358	EOS Hu03	432258	133	Hs.293039	ESTs	progression
359	EOS Hu03	432327	133	Hs.274363	neuroglobin	progression
360	EOS Hu03	432554	133	Hs.278411	NCK-associated protein 1	progression
361	EOS Hu03	432864	133	Hs.359682	calpastatin	progression
362	EOS Hu03	433052	133	Hs.293003	ESTs, Weakly similar to PC4259 ferritin associated protein [H.sapiens]	progression
363	EOS Hu03	433282	133	Hs.49007	hypothetical protein	progression
364	EOS Hu03	433844	133	Hs.179647	Homo sapiens cDNA FLJ12195 fis, clone MAMMA1000865	progression
365	EOS Hu03	433914	133	Hs.112160	Homo sapiens DNA helicase homolog (PIF1) mRNA, partial cds	progression
366	EOS Hu03	434055	133	Hs.3726	x 003 protein	progression
367	EOS Hu03	434263	133	Hs.79187	ESTs	progression
368	EOS Hu03	434547	133	Hs.106124	ESTs	progression
369	EOS Hu03	434831	133	Hs.273397	KIAA0710 gene product	progression
370	EOS Hu03	434978	133	Hs.4310	eukaryotic translation initiation factor 1A	progression
371	EOS Hu03	435158	133	Hs.65588	DAZ associated protein 1	progression
372	EOS Hu03	435320	133	Hs.117864	ESTs	progression
373	EOS Hu03	435521	133	Hs.6361	mitogen-activated protein kinase kinase 1 interacting protein 1	progression
374	EOS Hu03	436472	133	Hs.46366	KIAA0948 protein	progression
375	EOS Hu03	436576	133	Hs.77542	ESTs	progression
376	EOS Hu03	437223	133	Hs.330716	Homo sapiens cDNA FLJ14368 fis, clone HEMBA1001122	progression
377	EOS Hu03	437256	133	Hs.97871	Homo sapiens, clone IMAGE:3845253, mRNA, partial cds	progression
378	EOS Hu03	437524	133	Hs.385719	ESTs	progression
379	EOS Hu03	438013	133	Hs.15670	ESTs	progression
380	EOS Hu03	438644	133	Hs.129037	ESTs	progression

381	EOS Hu03	438818	133	Hs.30738	ESTs	progres-sion
382	EOS Hu03	438942	133	Hs.6451	PRO0659 protein	progres-sion
383	EOS Hu03	439010	133	Hs.75216	Homo sapiens cDNA FLJ13713 fis, clone PLACE2000398, moderately similar to LAR PROTEIN PRECURSOR (LEUKOCYTE ANTIGEN RELATED) (EC 3.1.3.48)	progres-sion
384	EOS Hu03	439130	133	Hs.375195	ESTs	progres-sion
385	EOS Hu03	439578	133	Hs.350547	nuclear receptor co-repressor/HDAC3 complex subunit	progres-sion
386	EOS Hu03	439632	133	Hs.334437	hypothetical protein MGC4248	progres-sion
387	EOS Hu03	440014	133	Hs.6856	ash2 (absent, small, or homeotic, Drosophila, homolog)-like	progres-sion
388	EOS Hu03	440100	133	Hs.158549	ESTs, Weakly similar to T2D3_HUMAN TRANSCRIPTION INITIATION FACTOR TFIID 135 KDA SUBUNIT [H.sapiens]	progres-sion
389	EOS Hu03	440197	133	Hs.317714	pallid (mouse) homolog, pallidin	progres-sion
390	EOS Hu03	440357	133	Hs.20950	phospholysine phosphohistidine inorganic pyrophosphate phosphatase	progres-sion
391	EOS Hu03	441650	133	Hs.132545	ESTs	progres-sion
392	EOS Hu03	442220	133	Hs.8148	selenoprotein T	progres-sion
393	EOS Hu03	442549	133	Hs.8375	TNF receptor-associated factor 4	progres-sion
394	EOS Hu03	443407	133	Hs.348514	ESTs, Moderately similar to 2109260A B cell growth factor [H.sapiens]	progres-sion
395	EOS Hu03	443471	133	Hs.398102	Homo sapiens clone FLB3442 PRO0872 mRNA, complete cds	progres-sion
396	EOS Hu03	443679	133	Hs.9670	hypothetical protein FLJ10948	progres-sion
397	EOS Hu03	443893	133	Hs.115472	ESTs, Weakly similar to 2004399A chromosomal protein [H.sapiens]	progres-sion
398	EOS Hu03	444037	133	Hs.380932	CHMP1.5 protein	progres-sion
399	EOS Hu03	444312	133	Hs.351142	ESTs	progres-sion
400	EOS Hu03	444336	133	Hs.10882	HMG-box containing protein 1	progres-sion
401	EOS Hu03	444604	133	Hs.11441	chromosome 1 open reading frame 8	progres-sion
402	EOS Hu03	445084	133	Hs.250848	hypothetical protein FLJ14761	progres-sion
403	EOS Hu03	445462	133	Hs.288649	hypothetical protein MGC3077	progres-sion
404	EOS Hu03	445692	133	Hs.182099	ESTs	progres-sion

405	EOS Hu03	445831	133	Hs.13351	LanC (bacterial lantibiotic synthetase component C)-like 1	progression
406	EOS Hu03	446556	133	Hs.15303	KIAA0349 protein	progression
407	EOS Hu03	446847	133	Hs.82845	Homo sapiens cDNA: FLJ21930 fis, clone HEP04301, highly similar to HSU90916 Human clone 23815 mRNA sequence	progression
408	EOS Hu03	447343	133	Hs.236894	ESTs, Highly similar to S02392 alpha-2-macroglobulin receptor precursor [H.sapiens]	progression
409	EOS Hu03	447400	133	Hs.18457	hypothetical protein FLJ20315	progression
410	EOS Hu03	448357	133	Hs.108923	RAB38, member RAS oncogene family	progression
411	EOS Hu03	448524	133	Hs.21356	hypothetical protein DKFZp762K2015	progression
412	EOS Hu03	448625	133	Hs.178470	hypothetical protein FLJ22662	progression
413	EOS Hu03	448780	133	Hs.267749	Human DNA sequence from clone 366N23 on chromosome 6q27. Contains two genes similar to consecutive parts of the C. elegans UNC-93 (protein 1, C46F11.1) gene, a KIAA0173 and Tubulin-Tyrosine Ligase LIKE gene, a Mitotic Feedback Control Protein MADP2 H	progression
414	EOS Hu03	448813	133	Hs.22142	cytochrome b5 reductase b5R.2	progression
415	EOS Hu03	449268	133	Hs.23412	hypothetical protein FLJ20160	progression
416	EOS Hu03	449626	133	Hs.112860	zinc finger protein 258	progression
417	EOS Hu03	450893	133	Hs.25625	hypothetical protein FLJ11323	progression
418	EOS Hu03	450997	133	Hs.35254	hypothetical protein FLB6421	progression
419	EOS Hu03	451164	133	Hs.60659	ESTs, Weakly similar to T46471 hypothetical protein DKFZp434L0130.1 [H.sapiens]	progression
420	EOS Hu03	451225	133	Hs.57655	ESTs	progression
421	EOS Hu03	451867	133	Hs.27192	hypothetical protein dJ1057B20.2	progression
422	EOS Hu03	451970	133	Hs.211046	ESTs	progression
423	EOS Hu03	452012	133	Hs.279766	kinesin family member 4A	progression
424	EOS Hu03	452170	133	Hs.28285	patched related protein translocated in renal cancer	progression
425	EOS Hu03	452517	133	-	gb:RC-BT068-130399-068 BT068 Homo sapiens cDNA, mRNA sequence	progression
426	EOS Hu03	452829	133	Hs.63368	ESTs, Weakly similar to TRHY_HUMAN TRICHOHYALI [H.sapiens]	progression
427	EOS Hu03	452929	133	Hs.172816	neuregulin 1	progression

428	EOS Hu03	453395	133	Hs.377915	mannosidase, alpha, class 2A, member 1	progression
429	EOS Hu03	454639	133		gb:RC2-ST0158-091099-011-d05 ST0158 Homo sapiens cDNA, mRNA sequence	progression
430	EOS Hu03	456332	133	Hs.399939	gb:nc39d05.r1 NCI_CGAP_Pr2 Homo sapiens cDNA clone, mRNA sequence	progression
431	EOS Hu03	457228	133	Hs.195471	Human cosmid CRI-JC2015 at D10S289 in 10sp13	progression
432	EOS Hu03	458132	133	Hs.103267	hypothetical protein FLJ22548 similar to gene trap PAT 12	progression
433	EOS Hu03	408688	133	Hs.152925	KIAA1268 protein	progression
434	EOS Hu03	410691	133	Hs.65450	reticulon 4	progression
435	EOS Hu03	420269	133	Hs.96264	alpha thalassemia/mental retardation syndrome X-linked (RAD54 (S. cerevisiae) homolog)	progression
436	EOS Hu03	422119	133	Hs.111862	KIAA0590 gene product	progression
437	EOS Hu03	422765	133	Hs.1578	baculoviral IAP repeat-containing 5 (survivin)	progression
438	EOS Hu03	422984	133	Hs.351597	ESTs	progression
439	EOS Hu03	428016	133	Hs.181461	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila)	progression
440	EOS Hu03	437325	133	Hs.5548	F-box and leucine-rich repeat protein 5	progression
441	EOS Hu03	444773	133	Hs.11923	hypothetical protein DJ167A19.1	progression
442	EOS Hu03	445926	133	Hs.334826	splicing factor 3b, subunit 1, 155kDa	progression
443	EOS Hu03	452714	133	Hs.30340	KIAA1165: likely ortholog of mouse Nedd4 WW domain-binding protein 5A	progression
444	EOS Hu03	452866	133	Hs.268016	ESTs	progression
445	EOS Hu03	453963	133	Hs.28959	cDNA FLJ36513 fis, clone TRACH2001523	progression
446	EOS Hu03	457329	133	Hs.359682	calpastatin	progression
447	U133A	200600_at	168	Hs.170328	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein	CIS
448	U133A	200762_at	168	Hs.173381	NM_019894; transmembrane protease, serine 4 isoform 1 NM_183247; transmembrane protease, serine 4 isoform 2	CIS
449	U133A	201088_at	168	Hs.159557	NM_000228; laminin subunit beta 3 precursor	CIS
450	U133A	201291_s_at	168	Hs.156346	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b	CIS
451	U133A	201560_at	168	Hs.25035	NM_005547; involucrin	CIS
452	U133A	201616_s_at	168	Hs.443811	NM_004692; NM_032727; internexin neuronal intermediate filament protein, alpha	CIS
453	U133A	201641_at	168	Hs.118110	NM_016233; peptidylarginine deiminase type III	CIS
454	U133A	201744_s_at	168	Hs.406475	NM_014417; BCL2 binding component 3	CIS

455	U133A	201842_s_at	168	Hs.76224	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog	CIS
456	U133A	201858_s_at	168	Hs.1908	NM_018058; cartilage acidic protein 1	CIS
457	U133A	201859_at	168	Hs.1908	NM_000497; cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 precursor	CIS
458	U133A	202746_at	168	Hs.17109	NM_007193; annexin A10	CIS
459	U133A	202917_s_at	168	Hs.416073	NM_001958; eukaryotic translation elongation factor 1 alpha 2	CIS
460	U133A	203009_at	168	Hs.155048	NM_005581; Lutheran blood group (Auberger b antigen included)	CIS
461	U133A	203287_at	168	Hs.18141	NM_005581; Lutheran blood group (Auberger b antigen included)	CIS
462	U133A	203477_at	168	Hs.409034	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b	CIS
463	U133A	203649_s_at	168	Hs.76422	NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)	CIS
464	U133A	203759_at	168	Hs.75268	NM_007193; annexin A10	CIS
465	U133A	203792_x_at	168	Hs.371617	NM_007144; ring finger protein 110	CIS
466	U133A	203842_s_at	168	Hs.172740	NM_014417; BCL2 binding component 3	CIS
467	U133A	203980_at	168	Hs.391561	NM_001442; fatty acid binding protein 4, adipocyte	CIS
468	U133A	204141_at	168	Hs.300701	NM_017689; hypothetical protein FLJ20151	CIS
469	U133A	204380_s_at	168	Hs.1420	NM_007144; ring finger protein 110	CIS
470	U133A	204465_s_at	168	Hs.76888	NM_004692; NM_032727; internexin neuronal intermediate filament protein, alpha	CIS
471	U133A	204487_s_at	168	Hs.367809	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3	CIS
472	U133A	204508_s_at	168	Hs.279916	NM_017689; hypothetical protein FLJ20151	CIS
473	U133A	204540_at	168	Hs.433839	NM_001958; eukaryotic translation elongation factor 1 alpha 2	CIS
474	U133A	204688_at	168	Hs.409798	NM_016233; peptidylarginine deiminase type III	CIS
475	U133A	204952_at	168	Hs.377028	NM_000445; plectin 1, intermediate filament binding protein 500kDa	CIS
476	U133A	204990_s_at	168	Hs.85266	NM_000213; integrin, beta 4	CIS
477	U133A	205073_at	168	Hs.152096	NM_019894; transmembrane protease, serine 4 isoform 1 NM_183247; transmembrane protease, serine 4 isoform 2	CIS
478	U133A	205382_s_at	168	Hs.155597	NM_000213; integrin, beta 4	CIS
479	U133A	205453_at	168	Hs.290432	NM_002145; homeo box B2	CIS
480	U133A	205455_at	168	Hs.2942	NM_006760; uroplakin 2	CIS
481	U133A	205927_s_at	168	Hs.1355	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein	CIS
482	U133A	206122_at	168	Hs.95582	NM_006942; SRY-box 15	CIS
483	U133A	206191_at	168	Hs.47042	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3	CIS
484	U133A	206392_s_at	168	Hs.82547	NM_005522; homeobox A1 protein isoform a NM_153620; homeobox A1 protein isoform b	CIS
485	U133A	206393_at	168	Hs.83760	NM_003282; troponin I, skeletal, fast	CIS
486	U133A	206465_at	168	Hs.277543	NM_015162; lipidosin	CIS
487	U133A	206561_s_at	168	Hs.116724	NM_015162; lipidosin	CIS
488	U133A	206658_at	168	Hs.284211	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b	CIS
489	U133A	207173_x_at	168	Hs.443435	NM_000213; integrin, beta 4	CIS
490	U133A	207862_at	168	Hs.379613	NM_006760; uroplakin 2	CIS
491	U133A	209138_x_at	168	Hs.505407	NM_015162; lipidosin	CIS
492	U133A	209270_at	168	Hs.436983	NM_000228; laminin subunit beta 3 precursor	CIS
493	U133A	209340_at	168	Hs.21293	NM_007144; ring finger protein 110	CIS
494	U133A	209591_s_at	168	Hs.170195	NM_000228; laminin subunit beta 3 precursor	CIS
495	U133A	209732_at	168	Hs.85201	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3	CIS
496	U133A	210143_at	168	Hs.188401	NM_007193; annexin A10	CIS
497	U133A	210735_s_at	168	Hs.5338	NM_017689; hypothetical protein FLJ20151	CIS
498	U133A	210761_s_at	168	Hs.86859	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog	CIS
499	U133A	211002_s_at	168	Hs.82237	NM_001958; eukaryotic translation elongation factor 1 alpha 2	CIS
500	U133A	211161_s_at	168		NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)	CIS

501	U133A	211430_s_at	168	Hs.413826	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein	CIS
502	U133A	211671_s_at	168	Hs.126608	NM_007144; ring finger protein 110	CIS
503	U133A	211692_s_at	168	Hs.87246	NM_014417; BCL2 binding component 3	CIS
504	U133A	211896_s_at	168	Hs.156316	NM_005581; Lutheran blood group (Auberger b antigen included)	CIS
505	U133A	212077_at	168	Hs.443811	NM_003282; troponin I, skeletal, fast	CIS
506	U133A	212192_at	168	Hs.109438	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog	CIS
507	U133A	212195_at	168	Hs.71968	NM_000445; plectin 1, intermediate filament binding protein 500kDa	CIS
508	U133A	212386_at	168	Hs.359289	NM_005547; involucrin	CIS
509	U133A	212667_at	168	Hs.111779	NM_000299; plakophilin 1	CIS
510	U133A	212671_s_at	168	Hs.387679	NM_002145; homeo box B2	CIS
511	U133A	212998_x_at	168	Hs.375115	NM_000497; cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 precursor	CIS
512	U133A	213891_s_at	168	Hs.359289	NM_007193; annexin A10	CIS
513	U133A	213975_s_at	168	Hs.234734	NM_005522; homeobox A1 protein isoform a NM_153620; homeobox A1 protein isoform b	CIS
514	U133A	214352_s_at	168	Hs.412107	NM_006760; uroplakin 2	CIS
515	U133A	214599_at	168	Hs.157091	NM_005547; involucrin	CIS
516	U133A	214630_at	168	Hs.184927	NM_000497; cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 precursor	CIS
517	U133A	214639_s_at	168	Hs.67397	NM_005522; homeobox A1 protein isoform a NM_153620; homeobox A1 protein isoform b	CIS
518	U133A	214651_s_at	168	Hs.127428	NM_002145; homeo box B2	CIS
519	U133A	214669_x_at	168	Hs.377975	NM_001442; fatty acid binding protein 4, adipocyte	CIS
520	U133A	214677_x_at	168	Hs.449601	NM_006942; SRY-box 15	CIS
521	U133A	214752_x_at	168	Hs.195464	NM_006942; SRY-box 15	CIS
522	U133A	215076_s_at	168	Hs.443625	NM_016233; peptidylarginine deiminase type III	CIS
523	U133A	215121_x_at	168	Hs.356861	NM_018058; cartilage acidic protein 1	CIS
524	U133A	215176_x_at	168	Hs.503443	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3	CIS
525	U133A	215379_x_at	168	Hs.449601	NM_006760; uroplakin 2	CIS
526	U133A	215812_s_at	168	Hs.499113	NM_018058; cartilage acidic protein 1	CIS
527	U133A	216641_s_at	168	Hs.18141	NM_005547; involucrin	CIS
528	U133A	216971_s_at	168	Hs.79706	NM_000445; plectin 1, intermediate filament binding protein 500kDa	CIS
529	U133A	217028_at	168	Hs.421986	NM_003282; troponin I, skeletal, fast	CIS
530	U133A	217040_x_at	168	Hs.95582	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein	CIS
531	U133A	217388_s_at	168	Hs.444471	NM_000228; laminin subunit beta 3 precursor	CIS
532	U133A	217626_at	168	Hs.201967	NM_000299; plakophilin 1	CIS
533	U133A	218484_at	168	Hs.221447	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog	CIS
534	U133A	218656_s_at	168	Hs.93765	NM_001442; fatty acid binding protein 4, adipocyte	CIS
535	U133A	218718_at	168	Hs.43080	NM_000445; plectin 1, intermediate filament binding protein 500kDa	CIS
536	U133A	218918_at	168	Hs.8910	NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)	CIS
537	U133A	218960_at	168	Hs.414005	NM_019894; transmembrane protease, serine 4 isoform 1 NM_183247; transmembrane protease, serine 4 isoform 2	CIS
538	U133A	219410_at	168	Hs.104800	NM_004692; NM_032727; internexin neuronal intermediate filament protein, alpha	CIS
539	U133A	219922_s_at	168	Hs.289019	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b	CIS
540	U133A	220026_at	168	Hs.227059	NM_001442; fatty acid binding protein 4, adipocyte	CIS
541	U133A	220779_at	168	Hs.149195	NM_016233; peptidylarginine deiminase type III	CIS
542	U133A	221204_s_at	168	Hs.326444	NM_018058; cartilage acidic protein 1	CIS
543	U133A	221660_at	168	Hs.247831	NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)	CIS
544	U133A	221671_x_at	168	Hs.377975	NM_000299; plakophilin 1	CIS

545	U133A	221854_at	168	Hs.313068	NM_000299; plakophilin 1	CIS
546	U133A	221872_at	168	Hs.82547	NM_001958; eukaryotic translation elongation factor 1 alpha 2	CIS
547	U133A	200958_s_at	168	Hs.164067	NM_005625; syndecan binding protein (syntenin)	CIS
548	U133A	201877_s_at	168	Hs.249955	NM_002719; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform a NM_178586; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform b NM_178587; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform c NM_178588; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform d	CIS
549	U133A	201887_at	168	Hs.285115	NM_001560; interleukin 13 receptor, alpha 1 precursor	CIS
550	U133A	202076_at	168	Hs.289107	NM_001166; baculoviral IAP repeat-containing protein 2	CIS
551	U133A	202777_at	168	Hs.104315	NM_007373; soc-2 suppressor of clear homolog	CIS
552	U133A	204640_s_at	168	Hs.129951	NM_003563; speckle-type POZ protein	CIS
553	U133A	209004_s_at	168	Hs.5548	NM_012161; F-box and leucine-rich repeat protein 5 isoform 1 NM_033535; F-box and leucine-rich repeat protein 5 isoform 2	CIS
554	U133A	209241_x_at	168	Hs.112028	NM_015716; misshapen/NIK-related kinase isoform 1 NM_153827; misshapen/NIK-related kinase isoform 3 NM_170663; misshapen/NIK-related kinase isoform 2	CIS
555	U133A	209579_s_at	168	Hs.35947	NM_003925; methyl-CpG binding domain protein 4	CIS
556	U133A	209630_s_at	168	Hs.444354	NM_012164; F-box and WD-40 domain protein 2	CIS
557	U133A	212784_at	168	Hs.388236	NM_015125; capicua homolog	CIS
558	U133A	212802_s_at	168	Hs.287266		CIS
559	U133A	212899_at	168	Hs.129836	NM_015076; cyclin-dependent kinase (CDC2-like) 11	CIS
560	U133A	213633_at	168	Hs.97858	NM_018957; SH3-domain binding protein 1	CIS
561	U133A	217941_s_at	168	Hs.8117	NM_018695; erbb2 interacting protein	CIS
562	U133A	218150_at	168	Hs.342849	NM_012097; ADP-ribosylation factor-like 5 isoform 1 NM_177985; ADP-ribosylation factor-like 5 isoform 2	CIS

The expression level of at least one gene in the sample is determined, wherein at least one of said genes is selected from the genes of Table A. The samples according to the present invention may be any tissue sample or body fluid sample, it is however often preferred to conduct the methods according to the invention on epithelial tissue, such as epithelial tissue from the bladder. In particular the epithelial tissue may be mucosa. In another embodiment the sample is a urine sample comprising the tissue cells.

The sample may be obtained by any suitable manner known to the man skilled in the art, such as a biopsy of the tissue, or a superficial sample scraped from the tissue. The sample may be prepared by forming a cell suspension made from the tissue, or by obtaining an extract from the tissue.

In one embodiment it is preferred that the sample comprises substantially only cells from said tissue, such as substantially only cells from mucosa of the bladder.

The methods according to the invention may be used for determining any biological condition, wherein said condition leads to a change in the expression of at least one gene, and preferably a change in a variety of genes.

Thus, the biological condition may be any malignant or premalignant condition, in particular in bladder, such as a tumor or an adenocarcinoma, a carcinoma, a teratoma, a sarcoma, and/or a lymphoma, and/or carcinoma-in-situ, and/or dysplasia-in-situ.

The expression level may be determined as single gene approaches, i.e. wherein the determination of expression from one or two or a few genes is conducted. It is however preferred that information is obtained from several genes, so that an expression pattern is obtained.

In a preferred embodiment expression from at least one gene from a first group is determined, said first gene group representing genes being expressed at a higher level in one type of tissue, i.e. tissue in one stage or one risk group, in combination with determination of expression of at least one gene from a second group, said second group representing genes being expressed at a higher level in tissue from another stage or from another risk group. Thereby the validity of the prediction increases, since expression levels from genes from more than one group are determined.

However, determination of the expression of a single gene whether belonging to the first group or second group is also within the scope of the present invention. In this case it is preferred that the single gene is selected among genes having a high change in expression level from normal cells to biological condition cells.

Another approach is determination of an expression pattern from a variety of genes, wherein the determination of the biological condition in the tissue relies on information from a variety of gene expression, i.e. rather on the combination of expressed genes than on the information from single genes.

The following data presented herein relates to bladder tumors, and therefore the description has focused on the gene expression level as one way of identifying genes that lose or gain function in cancer tissue. Genes showing a remarkable downregulation (or complete loss) or upregulation (gene expression gained de novo) of the expression level - measured as the mRNA transcript, during the malignant progression in bladder from normal mucosa through Ta superficial tumors, and Carcinoa in situ (CIS) to T1, slightly invasive tumors, to T2, T3 and T4 which have spread to muscle or even further into lymph nodes or other organs are within the scope of the invention, as well as genes gaining importance during the differentiation from normal towards malignancy.

The present invention relates to a variety of genes identified either by an EST identification number and/or by a gene identification number. Both type of identification numbers relates to identification numbers of UniGene database, NCBI, build 18.

The various genes have been identified using Affymetrix arrays of the following product numbers:
HUGeneFL (sold in 2000-2002)
EOS Hu03 (customized Affymetric array)
U133A (product #900367 sold in 2003)

Stage of a bladder tumor indicates how deep the tumor has penetrated. Superficial tumors are termed Ta, and Carcinoma in situ (CIS), and T1, T2, T3 and T4 are used to describe increasing degrees of penetration into the muscle. The grade of a bladder tumor is expressed on a scale of I-IV (1-4) according to Bergkvist, A.; ljungquist, A.; Moberger, B."Classification of bladder tumours basedf on the cellular pattern. Preliminary report of a clinical-pathological study of 300 cases with a minimum follow-up of eight years", Acta Chir Scand., 1965, 130(4):371-8). The grade reflects the cytological appearance of the cells. Grade I cells are almost normal. Grade II cells are slightly deviant. Grade III cells are clearly abnormal. And Grade IV cells are highly abnormal. A special form of bladder malignancy is carcinoma-in-situ or dyplasia-in-situ in which the altered cells are located in-situ.

It is important to predict the prognosis of a cancer disease, as superficial tumors may require a less intensive treatment than invasive tumors. According to the invention the expression level of genes may be used to identify genes whose expression can be used to identify a certain stage and/or the prognosis of the disease. These "Classifiers" are divided into those which can be used to identify Ta, Carcinoma in situ (CIS), T1, and T2 stages as well as those identifying risk of recurrence or progression. In one aspect of the invention measuring the transcript level of one or more of these genes may lead to a classifier that can add supplementary information to the information obtained from the pathological classification. For example gene expression levels that signify a T2 stage will be unfavourable to detect in a Ta tumor, as they may signal that the Ta tumor has the potential to become a T2 tumor. The opposite is probably also true, that an expression level that signify Ta will be favorable to have in a T2 tumor. In that way independent information may be obtained from pathological classification and a classification based on gene expression levels is made.

In the present context a standard expression level is the level of expression of a gene in a standard situation, such as a standard Ta tumor or a standard T2 tumor. For use in the present invention standard expression levels is determined for each stage as well as for each

group of progression, recurrence, and other prognostic indices. It is then possible to compare the result of a determination of the expression level from a gene of a given biological condition with a standard for each stage, progression, recurrence and other indices to obtain a classification of the biological condition.

Furthermore, in the present context a reference patterne refers to the pattern of expression levels seen in standard situations as discussed above, and reference patterns may be used as discussed above for standard expression levels.

It is known from the histopathological classification of bladder tumors that some information is obtained from merely classifying into stage and grade of tumor. Accordingly, in one aspect, the invention relates to a method of predicting the prognosis of the biological condition by determining the stage of the biological condition, by determining an expression level of at least one gene, wherein said gene is selected from the group of genes consisting of gene No 1 to gene No. 562. In this aspect information about the stage reveils directly information about the prognosis as well. An example hereof is when a bladder tumor is classified as for example stage T2, then the prognosis for the bladder tumor is obtained directly from the prognosis related generally to stage T2 tumors. In a preferred embodiment the genes for predicting the prognosis by establishing the stage of the tumor may be selected from gene selected from the group of genes consisting of gene No. 1 to gene No. 188. More preferably the genes for predicting the prognosis by establishing the stage of the tumor may be selected from gene selected from the group of genes consisting of gene Nos. 18, 39, 40, 55, 58, 79, 86, 87, 88, 91, 93, 103, 105, 106, 121, 123, 125, 126, 136, 137, 140, 149, 156, 158, 161, 165, 166, 167, 175, 184, 187, 188.

It is preferred that the expresison level of more one gene is determined, such as the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of at least six genes, such as the expression level of at least seven genes, such as the expression level of at least eight genes, such as the expression level of at least nine genes, such as the expression level of at least ten genes, such as the expression level of at least 15 genes, such as the expression level of at least 20 genes, such as the expression levels of at least 25 genes, such as the expression levels of at least 30 genes, such as the expression level of 32 genes.

As discussed above, in relation to bladder cancer the stages of a bladder tumor are selected from bladder cancer stages Ta, Carcinoma in situ, T1, T2, T3 and T4. In an embodiment the determination of a stage comprises

assaying at least the expression of Ta stage gene from a Ta stage gene group, at least one expression of a CIS gene, at least one expression of T1 stage gene from a T1 stage gene group, at least the expression of T2 stage gene from a T2 stage gene group, and more preferably assaying at least the expression of Ta stage gene from a Ta stage gene group, at least one expression of a CIS gene, at least one expression of T1 stage gene from a T1 stage gene group, at least the expression of T2 stage gene from a T2 stage gene group, at least the expression of T3 stage gene from a T3 stage gene group, at least the expression of T4 stage gene from a T4 stage gene group wherein at least one gene from each gene group is expressed in a significantly different amount in that stage than in one of the other stages.

Preferably, the genes selected may be a gene from each gene group being expressed in a significantly higher amount in that stage than in one of the other stages as compared to normal controls, see for example Table B below.

The genes selected may be a gene from each gene group being expressed in a significantly lower amount in that stage than in one of the other stages.

In another embodiment the present invention relates to a method of predicting the prognosis of a biological condition by obtaining information in addition to the stage classification as such. As described above, by determining gene expression levels that signify a T2 stage in a tumor otherwise classified as a Ta tumor, the expression levels signal that the Ta tumor has the potential to become a T2 tumor. The opposite is also true, that an expression level that signify Ta will be favorable to have in a T2 tumor. In the present invention the inventors have shown that some genes are relevant for obtaining this additional information.

Also, in one embodiment the present invention relates to a further method of predicting the prognosis of a biological condition by obtaining information in addition to the stage classification as such. Determination of squamous metaplasia in a tumor, in particular in a T2 stage tumor, is indicative of risk of progression. In particular the genes may be selected from gene selected from the group of genes consisting of gene No. 215 to gene No. 232, see also table H.

It is preferred that the expresison level of more one gene is determined, such as the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of at least six genes, such as the expression level of at least seven genes, such as the expression level of at least eight genes, such as the expression level of at least nine genes, such as the expression level of at least ten genes, such as the expression level of at least 15 genes, such as the expression level of 18 genes.

In another embodiment the invention relates to genes bearing information of recurrence of the biological condition as such. In particular the genes may be selected from gene selected from the group of genes consisting of gene No. 189 to gene No. 214. It is preferred to determine a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from the group of genes wherein expression is increased in case of recurrence, genes No. 189 to gene No. 199 (recurrence genes), and determined a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from the group of genes wherein expression is increased in case of no recurrence, genes No. 200 to No. 214 (non-recurrence genes), and correlate the first expression level to a standard expression level for progressors, and/or the second expression level to a standard expression level for non-progressors to predict the prognosis of the biological condition in the animal tissue, see also table C.

It is preferred that the expresison level of more one gene is determined, such as the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of at least six genes, such as the expression level of at least seven genes, such as the expression level of at least eight genes, such as the expression level of at least nine genes, such as the expression level of at least ten genes, such as the expression level of at least 15 genes, such as the expression level of at least 20 genes, such as the expression level of at least 25 genes, such as the expression level of 26 genes.

Furthermore, in another embodiment the invention relates to genes bearing information of progression as such. In particular the genes may be selected from the group of genes of table D, more preferably selected from the group of genes consisting of gene No. 233 to gene No. 446. More preferably the genes may be selected from the group of genes Nos. 255, 273, 279, 280, 281, 282 , 287, 295, 300, 311, 317, 320, 333, 346, 347, 349, 352, 364, 365, 373, 383, 386, 390, 394, 401 ,407, 414, 417, 426, 427, 428, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, see table E.

It is preferred that the expresison level of more one gene is determined, such as the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of at least six genes, such as the expression level of at least seven genes, such as the expression level of at least eight genes, such as the expression level of at least nine genes, such as the expression level of at least ten genes, such as the expression level of at least 15 genes, such as the expression level of at least 20 genes, such as the expression levels of at least 25 genes, such as the expression levels of at least

30 genes, such as the expression level of at least 35 genes, such as the expression level of at least 40 genes, such as the expression level of 45 genes.

Furthermore, it is within the scope of the invention to predict the prognosis of a biological condition in animal tissue by determining the expression level of at least two genes, by

determining a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from the group of gene Nos. 237, 238, 239, 240, 241, 242, 243, 245, 246, 247, 248, 250, 253, 254, 257, 258, 260, 263, 264, 265, 267, 270, 271, 272, 278, 283, 284, 287, 288, 290, 291, 292, 294, 297, 298, 300, 302, 303, 305, 309, 310, 315, 316, 317, 318, 319, 321, 324, 329, 335, 336, 337, 339, 340, 344, 346, 347, 354, 356, 358, 359, 362, 364, 365, 368, 369, 371, 372, 377, 378, 379, 380, 381, 382, 383, 384, 388, 391, 393, 395, 396, 397, 399, 402, 403, 404, 409, 413, 417, 419, 420, 421, 422, 423, 425, 427 ,429, 430, 431, 432, 437, 444 (progressor genes), and

determining a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from the group of genes Nos. 233, 234, 235, 236, 244, 249, 251, 252, 255, 256, 259, 261, 262, 266, 268, 269, 273, 274, 275, 276, 277, 279, 280, 281, 282, 285, 286, 289, 293, 295, 296, 299, 301, 304, 306, 307, 308, 311, 312, 313, 314 , 320 , 322, 323, 325, 326, 327, 328 , 330, 331, 332, 333, 334, 338, 341, 342, 343, 345, 348, 349, 350, 351, 352, 353, 355, 357, 360, 361, 363, 366, 367, 370, 373, 374, 375, 376, 385, 386, 387, 389, 390, 392, 394, 398, 400, 401, 405, 406, 407, 408, 410, 411, 412, 414, 415, 416, 418, 424, 426, 428, 433, 434, 435, 436, 438, 439, 440, 441, 442, 443, 445, 446 (non-progressor genes), and

correlating the first expression level to a standard expression level for progressors, and/or the second expression level to a standard expression level for non-progressors to predict the prognosis of the biological condition in the animal tissue.

In particular the genes of the first group and the second group for predicting the prognosis of a Ta stage tumor may be selected from gene selected from the group of progression/non-progession genes described above.

In yet another embodiment the present invention offers the possibility to predict the presence or absence of Carcinoma in situ in the same organ as the primary biological condition. An example hereof is for a Ta bladder tumor to predict, whether the bladder in addition to the Ta tumor comprises Carcinoma in situ (CIS). The presence of carcinoma in situ in a bladder containing a superficial Ta tumor is a signal that the Ta tumor has the potential of recurrence

and invasiveness. Accordingly, by predicting the presence of carcinoma in situ important information about the prognosis is obtained. In the present context, genes for predicting the presence of carcinoma in situ for a Ta stage tumor may be selected from gene selected from the group of genes consisting of gene No. 447 to gene No. 562. More preferably the genes are selected from the group of genes consisting of gene Nos 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480 ,481, 482, 483 ,484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495 , 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517 ,518 ,519, 520, 521, 522 ,523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, see table F, or from the group of genes consisting of gene Nos. 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, see table G.

It is preferred that the expresison level of more one gene is determined, such as the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of at least six genes, such as the expression level of at least seven genes, such as the expression level of at least eight genes, such as the expression level of at least nine genes, such as the expression level of at least ten genes, such as the expression level of at least 15 genes, such as the expression level of at least 20 genes, such as the expression levels of at least 25 genes, such as the expression levels of at least 30 genes, such as the expression level of at least 35 genes, such as the expression level of at least 40 genes, such as the expression level of at least 45 genes, such as the expression level of at least 50 genes, such as 100 genes. In another embodiment the expression level of 16 genes are determined.

It is also preferred to determine a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from the group of genes wherein expression is increased in case of CIS, genes Nos. 447, 448, 449, 450, 451, 452, 454, 455 ,456, 457, 458, 459, 462, 468, 474, 478, 484, 489, 491, 493, 495, 500, 501, 502, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 518 , 519, 520, 522, 523, 524, 525, 529, 531, 534, 535, 536, 538, 544, 546, 547, 548, 549, 550, 551, 552, 553, 555, 556, 558, 559, 561, 562 (CIS genes), and determined a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from the group of genes wherein expression is increased in case of no CIS, genes Nos. 453, 460, 461, 463, 464, 465, 466, 467, 469, 470, 471, 472, 473, 475, 476, 477, 479, 480, 481, 482, 483, 485, 486, 487, 488, 490, 492, 494, 496, 497, 498 , 499, 503, 515, 516, 517, 521, 526, 527, 528, 530 ,532, 533, 537, 539, 540, 541, 542, 543, 545, 554, 557, 560 (non-CIS genes), and correlate the first expression level to a standard expression level for CIS, and/or the second ex-

pression level to a standard expression level for non-CIS to predict the prognosis of the biological condition in the animal tissue.

It is preferred when determining the expression level of at least one gene from a first group and at least one gene from a second group that the expression level of more than one genes from each group is determined. Thus, it is preferred that the expresison level of more one gene is determined, such as the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of at least six genes, such as the expression level of at least seven genes, such as the expression level of at least eight genes, such as the expression level of at least nine genes, such as the expression level of at least ten genes in each group.

In one embodiment of the invention the stage of the biological condition has been determined before the prediction of prognosis. The stage may be determined by any suitable means such as determined by histological examination of the tissue or by genotyping of the tissue, preferably by genotyping of the tissue such as described herein or as described in WO 02/02804 incorporated herein by reference.

In another aspect the invention relates to a method of determining the stage of a biological condition in animal tissue,

comprising collecting a sample comprising cells from the tissue,

determining an expression level of at least one gene selected from the group of genes consisting of gene No. 1 to gene No. 562,

correlating the expression level of the assessed genes to at least one standard level of expression determining the stage of the condition.

In particular the expression level of at least one gene selected from the group of genes consisting of gene Nos. 1-457 and gene Nos. 459-535 and gene Nos. 537-562.

Specific embodiments of determining the stage is as described above for predicting prognosis by determination of stage.

In a preferred embodiment the expression level of at least two genes is determined by

determining the expression of at least a first stage gene from a first stage gene group and at least a second stage gene from a second stage gene group, wherein at least one of said genes is expressed in said first stage of the condition in a higher amount than in said second stage, and the other gene is a expressed in said first stage of the condition in a lower amount than in said second stage of the condition, and

correlating the expression level of the assessed genes to a standard level of expression determining the stage of the condition.

In general, genes being downregulated for higher stage tumors as well as for progression and recurrence may be of importance as predictive markers for the disease as loss of one or more of these may signal a poor outcome or an aggressive disease course. Furthermore, they may be important targets for therapy as restoring their expression level, e.g. by gene therapy, or substitution with those peptide products or small molecules with a similar biological effect may suppress the malignant growth.

Genes that are up-regulated (or gained de novo) during the malignant progression of bladder cancer from normal tissue through Ta, T1, T2, T3 and T4 is also within the scope of the invention. These genes are potential oncogenes and may be those genes that create or enhance the malignant growth of the cells. The expression level of these genes may serve as predictive markers for the disease course and treatment response, as a high level may signal an aggressive disease course, and they may serve as targets for therapy, as blocking these genes by e.g. anti-sense therapy, or by biochemical means could inhibit, or slow the tumor growth.

The genes used according to the invention show a sufficient difference in expression from one group to another and/or from one stage to another to use the gene as a classifier for the group and/or stage. Thus, comparison of an expression pattern to another may score a change from expressed to non-expressed, or the reverse. Alternatively, changes in intensity of expression may be scored, either increases or decreases. Any significant change can be used. Typical changes which are more than 2-fold are suitable. Changes which are greater than 5-fold are highly suitable.

The present invention in particular relates to methods using genes wherein at least a two-fold change in expression, such as at least a three-fold change, for example at least a four fold change, such as at least a five fold change, for example at least a six fold change, such as at least a ten fold change, for example at least a fifteen fold change, such as at least a twenty fold change is seen between two groups.

As described above the invention relates to the use of information of expression levels. In one embodiment the expression patterns is obtained, thus, the invention relates to a method of determining an expression pattern of a bladder cell sample, comprising:

collecting sample comprising bladder cells and/or expression products from bladder cells,

determining the expression level of at least one gene in the sample, said gene being selected from the group of genes consisting of gene No. 1 to gene No. 562, and obtaining an expression pattern of the bladder cell sample.

The invention preferably include more than one gene in the pattern, according it is preferred to include the expression level of at least two genes, such as the expression level of at least three genes, such as the expression level of at least four genes, such as the expression level of at least five genes, such as the expression level of more than six genes.

The expression pattern preferably relates to one or more of the group of genes discussed above with respect to prognosis relating to stage, SSC, progression, recurrence and/or CIS.

In order to predict prognosis and/or stages it is preferred to determine an expression pattern of a cell sample preferably independent of the proportion of submucosal, muscle and connective tissue cells present. Expression is determined of one or more genes in a sample comprising cells, said genes being selected from the same genes as discussed above and shown in the tables.

It is an object of the present invention that characteristic patterns of expression of genes can be used to characterize different types of tissue. Thus, for example gene expression patterns can be used to characterize stages and grades of bladder tumors. Similarly, gene expression patterns can be used to distinguish cells having a bladder origin from other cells. Moreover, gene expression of cells which routinely contaminate bladder tumor biopsies has been identified, and such gene expression can be removed or subtracted from patterns obtained from bladder biopsies. Further, the gene expression patterns of single-cell solutions of bladder tumor cells have been found to be substantially without interfering expression of contaminating muscle, submucosal, and connective tissue cells than biopsy samples.

The one or more genes exclude genes which are expressed in the submucosal, muscle, and connective tissue. A pattern of expression is formed for the sample which is independent of the proportion of submucosal, muscle, and connective tissue cells in the sample.

In another aspect of the invention a method of determining an expression pattern of a cell sample is provided. Expression is determined of one or more genes in a sample comprising cells. A first pattern of expression is thereby formed for the sample. Genes which are expressed in submucosal, muscle, and connective tissue cells are removed from the first pattern of expression, forming a second pattern of expression which is independent of the proportion of submucosal, muscle, and connective tissue cells in the sample.

Another embodiment of the invention provides a method for determining an expression pattern of a bladder mucosa or bladder cancer cell. Expression is determined of one or more genes in a sample comprising bladder mucosa or bladder cancer cells; the expression determined forms a first pattern of expression. A second pattern of expression which was formed using the one or more genes and a sample comprising predominantly submucosal, muscle, and connective tissue cells, is subtracted from the first pattern of expression, forming a third pattern of expression. The third pattern of expression reflects expression of the bladder mucosa or bladder cancer cells independent of the proportion of submucosal, muscle, and connective tissue cells present in the sample.

In one embodiment the invention provides a method to predict the prognosis of a bladder tumor as described above. A first pattern of expression is determined of one or more genes in a bladder tumor sample. The first pattern is compared to one or more reference patterns of expression determined for bladder tumors at different stages and/or in different groups. The reference pattern which shares maximum similarity with the first pattern is identified. The stage of the reference pattern with the maximum similarity is assigned to the bladder tumor sample.

Yet another embodiment the invention provides a method to determine the stage of a bladder tumor as described above. A first pattern of expression is determined of one or more genes in a bladder tumor sample. The first pattern is compared to one or more reference patterns of expression determined for bladder tumors at different stages. The reference pattern which shares maximum similarity with the first pattern is identified. The stage of the reference pattern with the maximum similarity is assigned to the bladder tumor sample.

Since a biopsy of the tissue often contains more tissue material such as connective tissue than the tissue to be examined, when the tissue to be examined is epithelial or mucosa, the invention also relates to methods, wherein the expression pattern of the tissue is independent of the amount of connective tissue in the sample.

Biopsies contain epithelial cells that most often are the targets for the studies, and in addition many other cells that contaminate the epithelial cell fraction to a varying extent. The

contaminants include histiocytes, endothelial cells, leukocytes, nerve cells, muscle cells etc. Micro dissection is the method of choice for DNA examination, but in the case of expression studies this procedure is difficult due to RNA degradation during the procedure. The epithelium may be gently removed and the expression in the remaining submucosa and underlying connective tissue (the bladder wall) monitored. Genes expressed at high or low levels in the bladder wall should be interrogated when performing expression monitoring of the mucosa and tumors. A similar approach could be used for studies of epithelia in other organs.

In one embodiment of the invention normal mucosa lining the bladder lumen from bladders for cancer is scraped off. Then biopsies is taken from the denuded submucosa and connective tissue, reaching approximately 5 mm into the bladder wall, and immediately disintegrated in guanidinium isothiocyanate. Total RNA may be extracted, pooled, and poly(A)$^+$ mRNA may be prepared from the pool followed by conversion to double-stranded cDNA and in vitro transcription into cRNA containing biotin-labeled CTP and UTP.

Genes that are expressed and genes that are not expressed in bladder wall can both interfere with the interpretation of the expression in a biopsy, and should be considered when interpreting expression intensities in tumor biopsies, as the bladder wall component of a biopsy varies in amount from biopsy to biopsy.

When having determined the pattern of genes expressed in bladder wall components said pattern may be subtracted from a pattern obtained from the sample resulting in a third pattern related to the mucosa (epithelial) cells.

In another embodiment of the invention a method is provided for determining an expression pattern of a bladder tissue sample independent of the proportion of submucosal, muscle and connective tissue cells present. A single-cell suspension of disaggregated bladder tumor cells is isolated from a bladder tissue sample comprising bladder tumor cells is isolated from a bladder tissue sample comprising bladder cells, submucosal cells, muscle cells, and connective tissue cells. A pattern of expression is thus formed for the sample which is independent of the proportion of submucosal, muscle, and connective tissue cells in the bladder tissue sample.

Yet another method relates to the elimination of mRNA from bladder wall components before determining the pattern, e.g. by filtration and/or affinity chromatography to remove mRNA related to the bladder wall.

Working with tumor material requires biopsies or body fluids suspected to comprise relevant cells. Working with RNA requires freshly frozen or immediately processed biopsies, or

chemical pretreatment of the biopsy. Apart from the cancer tissue, biopsies do inevitably contain many different cell types, such as cells present in the blood, connective and muscle tissue, endothelium etc. In the case of DNA studies, microdissection or laser capture are methods of choice, however the time-dependent degradation of RNA makes it difficult to perform manipulation of the tissue for more than a few minutes. Furthermore, studies of expressed sequences may be difficult on the few cells obtained via microdissection or laser capture, as these cells may have an expression pattern that deviates from the predominant pattern in a tumor due to large intratumoral heterogeneity.

In the present context high density expression arrays may be used to evaluate the impact of bladder wall components in bladder tumor biopsies, and tested preparation of single cell solutions as a means of eliminating the contaminants. The results of these evaluations permit for the design of methods of evaluating bladder samples without the interfering background noise caused by ubiquitous contaminating submucosal, muscle, and connective tissue cells. The evaluating assays of the invention may be of any type.

While high density expression arrays can be used, other techniques are also contemplated. These include other techniques for assaying for specific mRNA species, including RT-PCR and Northern Blotting, as well as techniques for assaying for particular protein products, such as ELISA, Western blotting, and enzyme assays. Gene expression patterns according to the present invention are determined by measuring any gene product of a particular gene, including mRNA and protein. A pattern may be for one or more genes.

RNA or protein can be isolated and assayed from a test sample using any techniques known in the art. They can for example be isolated from a fresh or frozen biopsy, from formalin-fixed tissue, from body fluids, such as blood, plasma, serum, urine, or sputum.

Expression of genes may in general be detected by either detecting mRNA from the cells and/or detecting expression products, such as peptides and proteins.

The detection of mRNA of the invention may be a tool for determining the developmental stage of a cell type which may be definable by its pattern of expression of messenger RNA. For example, in particular stages of cells, high levels of ribosomal RNA are found whereas relatively low levels of other types of messenger RNAs may be found. Where a pattern is shown to be characteristic of a stage, said stage may be defined by that particular pattern of messenger RNA expression. The mRNA population is a good determinant of a developmental stage, and may be correlated with other structural features of the cell. In this manner, cells at specific developmental stages will be characterized by the intracellular environment, as well as the extracellular environment. The present invention also allows the

combination of definitions based in part upon antigens and in part upon mRNA expression. In one embodiment, the two may be combined in a single incubation step. A particular incubation condition may be found which is compatible with both hybridization recognition and non-hybridization recognition molecules. Thus, e.g. an incubation condition may be selected which allows both specificity of antibody binding and specificity of nucleic acid hybridization. This allows simultaneous performance of both types of interactions on a single matrix. Again, where developmental mRNA patterns are correlated with structural features, or with probes which are able to hybridize to intracellular mRNA populations, a cell sorter may be used to sort specifically those cells having desired mRNA population patterns.

It is within the general scope of the present invention to provide methods for the detection of mRNA. Such methods often involve sample extraction, PCR amplification, nucleic acid fragmentation and labeling, extension reactions, and transcription reactions.

The nucleic acid (either genomic DNA or mRNA) may be isolated from the sample according to any of a number of methods well known to those of skill in the art. One of skill will appreciate that where alterations in the copy number of a gene are to be detected genomic DNA is preferably isolated. Conversely, where expression levels of a gene or genes are to be detected, preferably RNA (mRNA) is isolated.

Methods of isolating total mRNA are well known to those of skill in the art. In one embodiment, the total nucleic acid is isolated from a given sample using, for example, an acid guanidinium-phenol-chloroform extraction method and polyA.sup. and mRNA is isolated by oligo dT column chromatography or by using (dT)n magnetic beads (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989), or Current Protocols in Molecular Biology, F. Ausubel et al., ed. Greene Publishing and Wiley-Interscience, New York (1987)).

The sample may be from tissue and/or body fluids, as defined elsewhere herein. Before analyzing the sample, e.g., on an oligonucleotide array, it will often be desirable to perform one or more sample preparation operations upon the sample. Typically, these sample preparation operations will include such manipulations as extraction of intracellular material, e.g., nucleic acids from whole cell samples, viruses, amplification of nucleic acids, fragmentation, transcription, labeling and/or extension reactions. One or more of these various operations may be readily incorporated into the device of the present invention.

DNA extraction may be relevant under circumstances where possible mutations in the genes are to be determined in addition to the determination of expression of the genes.

For those embodiments where whole cells, or other tissue samples are being analyzed, it will typically be necessary to extract the nucleic acids from the cells or viruses, prior to continuing with the various sample preparation operations. Accordingly, following sample collection, nucleic acids may be liberated from the collected cells, viral coat etc. into a crude extract followed by additional treatments to prepare the sample for subsequent operations, such as denaturation of contaminating (DNA binding) proteins, purification, filtration and desalting.

Liberation of nucleic acids from the sample cells, and denaturation of DNA binding proteins may generally be performed by physical or chemical methods. For example, chemical methods generally employ lysing agents to disrupt the cells and extract the nucleic acids from the cells, followed by treatment of the extract with chaotropic salts such as guanidinium isothiocyanate or urea to denature any contaminating and potentially interfering proteins.

Alternatively, physical methods may be used to extract the nucleic acids and denature DNA binding proteins, such as physical protrusions within microchannels or sharp edged particles piercing cell membranes and extract their contents. Combinations of such structures with piezoelectric elements for agitation can provide suitable shear forces for lysis.

More traditional methods of cell extraction may also be used, e.g., employing a channel with restricted cross-sectional dimension which causes cell lysis when the sample is passed through the channel with sufficient flow pressure. Alternatively, cell extraction and denaturing of contaminating proteins may be carried out by applying an alternating electrical current to the sample. More specifically, the sample of cells is flowed through a microtubular array while an alternating electric current is applied across the fluid flow. Subjecting cells to ultrasonic agitation, or forcing cells through microgeometry apertures, thereby subjecting the cells to high shear stress resulting in rupture are also possible extraction methods.

Following extraction, it will often be desirable to separate the nucleic acids from other elements of the crude extract, e.g. denatured proteins, cell membrane particles and salts. Removal of particulate matter is generally accomplished by filtration or flocculation. Further, where chemical denaturing methods are used, it may be desirable to desalt the sample prior to proceeding to the next step. Desalting of the sample and isolation of the nucleic acid may generally be carried out in a single step, e.g. by binding the nucleic acids to a solid phase and washing away the contaminating salts, or performing gel filtration chromatography on the sample passing salts through dialysis membranes. Suitable solid supports for nucleic acid binding include e.g. diatomaceous earth or silica (i.e., glass wool). Suitable gel exclusion media also well known in the art may be readily incorporated into the devices of

the present invention and is commercially available from, e.g., Pharmacia and Sigma Chemical.

Alternatively, desalting methods may generally take advantage of the high electrophoretic mobility and negativity of DNA compared to other elements. Electrophoretic methods may also be utilized in the purification of nucleic acids from other cell contaminants and debris. Upon application of an appropriate electric field, the nucleic acids present in the sample will migrate toward the positive electrode and become trapped on the capture membrane. Sample impurities remaining free of the membrane are then washed away by applying an appropriate fluid flow. Upon reversal of the voltage, the nucleic acids are released from the membrane in a substantially purer form. Further, coarse filters may also be overlaid on the barriers to avoid any fouling of the barriers by particulate matter, proteins or nucleic acids, thereby permitting repeated use.

In a similar aspect, the high electrophoretic mobility of nucleic acids with their negative charges, may be utilized to separate nucleic acids from contaminants by utilizing a short column of a gel or other appropriate matrices or gels which will slow or retard the flow of other contaminants while allowing the faster nucleic acids to pass.

This invention provides nucleic acid affinity matrices that bear a large number of different nucleic acid affinity ligands allowing the simultaneous selection and removal of a large number of preselected nucleic acids from the sample. Methods of producing such affinity matrices are also provided. In general the methods involve the steps of a) providing a nucleic acid amplification template array comprising a surface to which are attached at least 50 oligonucleotides having different nucleic acid sequences, and wherein each different oligonucleotide is localized in a predetermined region of said surface, the density of said oligonucleotides is greater than about 60 different oligonucleotides per 1 cm.sup.2, and all of said different oligonucleotides have an identical terminal 3' nucleic acid sequence and an identical terminal 5' nucleic acid sequence. b) amplifying said multiplicity of oligonucleotides to provide a pool of amplified nucleic acids; and c) attaching the pool of nucleic acids to a solid support.

For example, nucleic acid affinity chromatography is based on the tendency of complementary, single-stranded nucleic acids to form a double-stranded or duplex structure through complementary base pairing. A nucleic acid (either DNA or RNA) can easily be attached to a solid substrate (matrix) where it acts as an immobilized ligand that interacts with and forms duplexes with complementary nucleic acids present in a solution contacted to the immobilized ligand. Unbound components can be washed away from the bound complex to either provide a solution lacking the target molecules bound to the affinity column, or to

provide the isolated target molecules themselves. The nucleic acids captured in a hybrid duplex can be separated and released from the affinity matrix by denaturation either through heat, adjustment of salt concentration, or the use of a destabilizing agent such as formamide, TWEEN.TM.-20 denaturing agent, or sodium dodecyl sulfate (SDS).

Affinity columns (matrices) are typically used either to isolate a single nucleic acid typically by providing a single species of affinity ligand. Alternatively, affinity columns bearing a single affinity ligand (e.g. oligo dt columns) have been used to isolate a multiplicity of nucleic acids where the nucleic acids all share a common sequence (e.g. a polyA).

The type of affinity matrix used depends on the purpose of the analysis. For example, where it is desired to analyze mRNA expression levels of particular genes in a complex nucleic acid sample (e.g., total mRNA) it is often desirable to eliminate nucleic acids produced by genes that are constitutively overexpressed and thereby tend to mask gene products expressed at characteristically lower levels. Thus, in one embodiment, the affinity matrix can be used to remove a number of preselected gene products (e.g., actin, GAPDH, etc.). This is accomplished by providing an affinity matrix bearing nucleic acid affinity ligands complementary to the gene products (e.g., mRNAs or nucleic acids derived therefrom) or to subsequences thereof. Hybridization of the nucleic acid sample to the affinity matrix will result in duplex formation between the affinity ligands and their target nucleic acids. Upon elution of the sample from the affinity matrix, the matrix will retain the duplexes nucleic acids leaving a sample depleted of the overexpressed target nucleic acids.

The affinity matrix can also be used to identify unknown mRNAs or cDNAs in a sample. Where the affinity matrix contains nucleic acids complementary to every known gene (e.g., in a cDNA library, DNA reverse transcribed from an mRNA, mRNA used directly or amplified, or polymerized from a DNA template) in a sample, capture of the known nucleic acids by the affinity matrix leaves a sample enriched for those nucleic acid sequences that are unknown. In effect, the affinity matrix is used to perform a subtractive hybridization to isolate unknown nucleic acid sequences. The remaining "unknown" sequences can then be purified and sequenced according to standard methods.

The affinity matrix can also be used to capture (isolate) and thereby purify unknown nucleic acid sequences. For example, an affinity matrix can be prepared that contains nucleic acid (affinity ligands) that are complementary to sequences not previously identified, or not previously known to be expressed in a particular nucleic acid sample. The sample is then hybridized to the affinity matrix and those sequences that are retained on the affinity matrix are "unknown" nucleic acids. The retained nucleic acids can be eluted from the matrix (e.g.

at increased temperature, increased destabilizing agent concentration, or decreased salt) and the nucleic acids can then be sequenced according to standard methods.

Similarly, the affinity matrix can be used to efficiently capture (isolate) a number of known nucleic acid sequences. Again, the matrix is prepared bearing nucleic acids complementary to those nucleic acids it is desired to isolate. The sample is contacted to the matrix under conditions where the complementary nucleic acid sequences hybridize to the affinity ligands in the matrix. The non-hybridized material is washed off the matrix leaving the desired sequences bound. The hybrid duplexes are then denatured providing a pool of the isolated nucleic acids. The different nucleic acids in the pool can be subsequently separated according to standard methods (e.g. gel electrophoresis).

As indicated above the affinity matrices can be used to selectively remove nucleic acids from virtually any sample containing nucleic acids (e.g. in a cDNA library, DNA reverse transcribed from an mRNA, mRNA used directly or amplified, or polymerized from a DNA template, and so forth). The nucleic acids adhering to the column can be removed by washing with a low salt concentration buffer, a buffer containing a destabilizing agent such as formamide, or by elevating the column temperature.

In one particularly preferred embodiment, the affinity matrix can be used in a method to enrich a sample for unknown RNA sequences (e.g. expressed sequence tags (ESTs)). The method involves first providing an affinity matrix bearing a library of oligonucleotide probes specific to known RNA (e.g., EST) sequences. Then, RNA from undifferentiated and/or unactivated cells and RNA from differentiated or activated or pathological (e.g., transformed) or otherwise having a different metabolic state are separately hybridized against the affinity matrices to provide two pools of RNAs lacking the known RNA sequences.

In a preferred embodiment, the affinity matrix is packed into a columnar casing. The sample is then applied to the affinity matrix (e.g. injected onto a column or applied to a column by a pump such as a sampling pump driven by an autosampler). The affinity matrix (e.g. affinity column) bearing the sample is subjected to conditions under which the nucleic acid probes comprising the affinity matrix hybridize specifically with complementary target nucleic acids. Such conditions are accomplished by maintaining appropriate pH, salt and temperature conditions to facilitate hybridization as discussed above.

For a number of applications, it may be desirable to extract and separate messenger RNA from cells, cellular debris, and other contaminants. As such, the device of the present invention may, in some cases, include a mRNA purification chamber or channel. In general, such purification takes advantage of the poly-A tails on mRNA. In particular and as noted

above, poly- T oligonucleotides may be immobilized within a chamber or channel of the device to serve as affinity ligands for mRNA. Poly-T oligonucleotides may be immobilized upon a solid support incorporated within the chamber or channel, or alternatively, may be immobilized upon the surface(s) of the chamber or channel itself. Immobilization of oligonucleotides on the surface of the chambers or channels may be carried out by methods described herein including, e.g., oxidation and silanation of the surface followed by standard DMT synthesis of the oligonucleotides.

In operation, the lysed sample is introduced to a high salt solution to increase the ionic strength for hybridization, whereupon the mRNA will hybridize to the immobilized poly-T. The mRNA bound to the immobilized poly-T oligonucleotides is then washed free in a low ionic strength buffer. The poy-T oligonucleotides may be immobiliized upon poroussurfaces, e.g., porous silicon, zeolites silica xerogels, scintered particles, or other solid supports.

Following sample preparation, the sample can be subjected to one or more different analysis operations. A variety of analysis operations may generally be performed, including size based analysis using, e.g., microcapillary electrophoresis, and/or sequence based analysis using, e.g., hybridization to an oligonucleotide array.

In the latter case, the nucleic acid sample may be probed using an array of oligonucleotide probes. Oligonucleotide arrays generally include a substrate having a large number of positionally distinct oligonucleotide probes attached to the substrate. These arrays may be produced using mechanical or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis methods.

The basic strategy for light directed synthesis of oligonucleotide arrays is as follows. The surface of a solid support, modified with photosensitive protecting groups is illuminated through a photolithographic mask, yielding reactive hydroxyl groups in the illuminated regions. A selected nucleotide, typically in the form of a 3'-O-phosphoramidite-activated deoxynucleoside (protected at the 5' hydroxyl with a photosensitive protecting group), is then presented to the surface and coupling occurs at the sites that were exposed to light. Following capping and oxidation, the substrate is rinsed and the surface is illuminated through a second mask to expose additional hydroxyl groups for coupling. A second selected nucleotide (e.g., 5'-protected, 3'-O-phosphoramidite-activated deoxynucleoside) is presented to the surface. The selective deprotection and coupling cycles are repeated until the desired set of products is obtained. Since photolithography is used the process can be readily miniaturized to generate high density arrays of oligonucleotide probes. Furthermore, the sequence of the oligonucleotides at each site is known. See Pease et al. Mechanical

synthesis methods are similar to the light directed methods except involving mechanical direction of fluids for deprotection and addition in the synthesis steps.

For some embodiments, oligonucleotide arrays may be prepared having all possible probes of a given length. The hybridization pattern of the target sequence on the array may be used to reconstruct the target DNA sequence. Hybridization analysis of large numbers of probes can be used to sequence long stretches of DNA or provide an oligonucleotide array which is specific and complementary to a particular nucleic acid sequence. For example, in particularly preferred aspects, the oligonucleotide array will contain oligonucleotide probes which are complementary to specific target sequences, and individual or multiple mutations of these. Such arrays are particularly useful in the diagnosis of specific disorders which are characterized by the presence of a particular nucleic acid sequence.

Following sample collection and nucleic acid extraction, the nucleic acid portion of the sample is typically subjected to one or more preparative reactions. These preparative reactions include in vitro transcription, labeling, fragmentation, amplification and other reactions. Nucleic acid amplification increases the number of copies of the target nucleic acid sequence of interest. A variety of amplification methods are suitable for use in the methods and device of the present invention, including for example, the polymerase chain reaction method or (PCR), the ligase chain reaction (LCR), self sustained sequence replication (3SR), and nucleic acid based sequence amplification (NASBA).

The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of approximately 30 or 100 to 1, respectively. As a result, where these latter methods are employed, sequence analysis may be carried out using either type of substrate, i.e. complementary to either DNA or RNA.

Frequently, it is desirable to amplify the nucleic acid sample prior to hybridization. One of skill in the art will appreciate that whatever amplification method is used, if a quantitative result is desired, care must be taken to use a method that maintains or controls for the relative frequencies of the amplified nucleic acids.

PCR

Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. The high density array may then include probes specific to the internal standard for quantification of the amplified nucleic acid.

Thus, in one embodiment, this invention provides for a method of optimizing a probe set for detection of a particular gene. Generally, this method involves providing a high density array containing a multiplicity of probes of one or more particular length(s) that are complementary to subsequences of the mRNA transcribed by the target gene. In one embodiment the high density array may contain every probe of a particular length that is complementary to a particular mRNA. The probes of the high density array are then hybridized with their target nucleic acid alone and then hybridized with a high complexity, high concentration nucleic acid sample that does not contain the targets complementary to the probes. Thus, for example, where the target nucleic acid is an RNA, the probes are first hybridized with their target nucleic acid alone and then hybridized with RNA made from a cDNA library (e.g., reverse transcribed polyA.sup.+ mRNA) where the sense of the hybridized RNA is opposite that of the target nucleic acid (to insure that the high complexity sample does not contain targets for the probes). Those probes that show a strong hybridization signal with their target and little or no cross-hybridization with the high complexity sample are preferred probes for use in the high density arrays of this invention.

PCR amplification generally involves the use of one strand of the target nucleic acid sequence as a template for producing a large number of complements to that sequence. Generally, two primer sequences complementary to different ends of a segment of the complementary strands of the target sequence hybridize with their respective strands of the target sequence, and in the presence of polymerase enzymes and nucleoside triphosphates, the primers are extended along the target sequence. The extensions are melted from the target sequence and the process is repeated, this time with the additional copies of the target sequence synthesized in the preceding steps. PCR amplification typically involves repeated cycles of denaturation, hybridization and extension reactions to produce sufficient amounts of the target nucleic acid. The first step of each cycle of the PCR involves the separation of the nucleic acid duplex formed by the primer extension. Once the strands are separated, the next step in PCR involves hybridizing the separated strands with primers that flank the target sequence. The primers are then extended to form complementary copies of the target strands. For successful PCR amplification, the primers are designed so that the position at which each primer hybridizes along a duplex sequence is such that an extension product synthesized from one primer, when separated from the template (complement), serves as a template for the extension of the other primer. The cycle of denaturation, hybridization, and extension is repeated as many times as necessary to obtain the desired amount of amplified nucleic acid.

In PCR methods, strand separation is normally achieved by heating the reaction to a sufficiently high temperature for a sufficient time to cause the denaturation of the duplex but

not to cause an irreversible denaturation of the polymerase. Typical heat denaturation involves temperatures ranging from about 80.degree. C. to 105.degree. C. for times ranging from seconds to minutes. Strand separation, however, can be accomplished by any suitable denaturing method including physical, chemical, or enzymatic means. Strand separation may be induced by a helicase, for example, or an enzyme capable of exhibiting helicase activity.

In addition to PCR and IVT reactions, the methods and devices of the present invention are also applicable to a number of other reaction types, e.g., reverse transcription, nick translation, and the like.

The nucleic acids in a sample will generally be labeled to facilitate detection in subsequent steps. Labeling may be carried out during the amplification, in vitro transcription or nick translation processes. In particular, amplification, in vitro transcription or nick translation may incorporate a label into the amplified or transcribed sequence, either through the use of labeled primers or the incorporation of labeled dNTPs into the amplified sequence.
Hybridization between the sample nucleic acid and the oligonucleotide probes upon the array is then detected, using, e.g., epifluorescence confocal microscopy. Typically, sample is mixed during hybridization to enhance hybridization of nucleic acids in the sample to nucleoc acid probes on the array.

In some cases, hybridized oligonucleotides may be labeled following hybridization. For example, where biotin labeled dNTPs are used in, e.g. amplification or transcription, streptavidin linked reporter groups may be used to label hybridized complexes. Such operations are readily integratable into the systems of the present invention. Alternatively, the nucleic acids in the sample may be labeled following amplification. Post amplification labeling typically involves the covalent attachment of a particular detectable group upon the amplified sequences. Suitable labels or detectable groups include a variety of fluorescent or radioactive labeling groups well known in the art. These labels may also be coupled to the sequences using methods that are well known in the art.

Methods for detection depend upon the label selected. A fluorescent label is preferred because of its extreme sensitivity and simplicity. Standard labeling procedures are used to determine the positions where interactions between a sequence and a reagent take place. For example, if a target sequence is labeled and exposed to a matrix of different probes, only those locations where probes do interact with the target will exhibit any signal. Alternatively, other methods may be used to scan the matrix to determine where interaction takes place. Of course, the spectrum of interactions may be determined in a temporal manner by repeated scans of interactions which occur at each of a multiplicity of conditions. However,

instead of testing each individual interaction separately, a multiplicity of sequence interactions may be simultaneously determined on a matrix.

Means of detecting labeled target (sample) nucleic acids hybridized to the probes of the high density array are known to those of skill in the art. Thus, for example, where a colorimetric label is used, simple visualization of the label is sufficient. Where a radioactive labeled probe is used, detection of the radiation (e.g with photographic film or a solid state detector) is sufficient.

In a preferred embodiment, however, the target nucleic acids are labeled with a fluorescent label and the localization of the label on the probe array is accomplished with fluorescent microscopy. The hybridized array is excited with a light source at the excitation wavelength of the particular fluorescent label and the resulting fluorescence at the emission wavelength is detected. In a particularly preferred embodiment, the excitation light source is a laser appropriate for the excitation of the fluorescent label.

The target polynucleotide may be labeled by any of a number of convenient detectable markers. A fluorescent label is preferred because it provides a very strong signal with low background. It is also optically detectable at high resolution and sensitivity through a quick scanning procedure. Other potential labeling moieties include, radioisotopes, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers, magnetic labels, and linked enzymes.
Another method for labeling may bypass any label of the target sequence. The target may be exposed to the probes, and a double strand hybrid is formed at those positions only. Addition of a double strand specific reagent will detect where hybridization takes place. An intercalative dye such as ethidium bromide may be used as long as the probes themselves do not fold back on themselves to a significant extent forming hairpin loops. However, the length of the hairpin loops in short oligonucleotide probes would typically be insufficient to form a stable duplex.

Suitable chromogens will include molecules and compounds which absorb light in a distinctive range of wavelengths so that a color may be observed, or emit light when irradiated with radiation of a particular wave length or wave length range, e.g., fluorescers. Biliproteins, e.g., phycoerythrin, may also serve as labels.

A wide variety of suitable dyes are available, being primarily chosen to provide an intense color with minimal absorption by their surroundings. Illustrative dye types include quinoline dyes, triarylmethane dyes, acridine dyes, alizarine dyes, phthaleins, insect dyes, azo dyes, anthraquinoid dyes, cyanine dyes, phenazathionium dyes, and phenazoxonium dyes.

A wide variety of fluorescers may be employed either by themselves or in conjunction with quencher molecules. Fluorescers of interest fall into a variety of categories having certain primary functionalities. These primary functionalities include 1- and 2-aminonaphthalene, p,p'-diaminostilbenes, pyrenes, quaternary phenanthridine salts, 9-aminoacridines, p,p'-diaminobenzophenone imines, anthracenes, oxacarbocyanine, merocyanine, 3-aminoequilenin, perylene, bis-benzoxazole, bis-p-oxazolyl benzene, 1,2-benzophenazin, retinol, bis-3-aminopyridinium salts, hellebrigenin, tetracycline, sterophenol, benzimidzaolylphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, salicylate, strophanthidin, porphyrins, triarylmethanes and flavin. Individual fluorescent compounds which have functionalities for linking or which can be modified to incorporate such functionalities include, e.g., dansyl chloride; fluoresceins such as 3,6-dihydroxy-9-phenylxanthhydrol; rhodamineisothiocyanate; N-phenyl 1-amino-8-sulfonatonaphthalene; N-phenyl 2-amino-6-sulfonatonaphthalene; 4-acetamido-4-isothiocyanato-stilbene-2,2'-disulfonic acid; pyrene-3-sulfonic acid; 2-toluidinonaphthalene-6-sulfonate; N-phenyl, N-methyl 2-aminoaphthalene-6-sulfonate; ethidium bromide; stebrine; auromine-0,2-(9'-anthroyl)palmitate; dansyl phosphatidylethanolamine; N,N'-dioctadecyl oxacarbocyanine; N,N'-dihexyl oxacarbocyanine; merocyanine, 4-(3'pyrenyl)butyrate; d-3-aminodesoxy-equilenin; 12-(9'-anthroyl)stearate; 2-methylanthracene; 9-vinylanthracene; 2,2'-(vinylene-p-phenylene)bisbenzoxazole; p-bis›2-(4-methyl-5-phenyl-oxazolyl)!benzene; 6-dimethylamino-1,2-benzophenazin; retinol; bis(3'-aminopyridinium) 1,10-decandiyl diiodide; sulfonaphthylhydrazone of hellibrienin; chlorotetracycline; N-(7-dimethylamino-4-methyl-2-oxo-3-chromenyl)maleimide; N-›p-(2-benzimidazolyl)-phenyl!maleimide; N-(4-fluoranthyl)maleimide; bis(homovanillic acid); resazarin; 4-chloro-7-nitro-2,1,3-benzooxadiazole; merocyanine 540; resorufin; rose bengal; and 2,4-diphenyl-3(2H)-furanone.

Desirably, fluorescers should absorb light above about 300 nm, preferably about 350 nm, and more preferably above about 400 nm, usually emitting at wavelengths greater than about 10 nm higher than the wavelength of the light absorbed. It should be noted that the absorption and emission characteristics of the bound dye may differ from the unbound dye. Therefore, when referring to the various wavelength ranges and characteristics of the dyes, it is intended to indicate the dyes as employed and not the dye which is unconjugated and characterized in an arbitrary solvent.

Fluorescers are generally preferred because by irradiating a fluorescer with light, one can obtain a plurality of emissions. Thus, a single label can provide for a plurality of measurable events.

Detectable signal may also be provided by chemiluminescent and bioluminescent sources. Chemiluminescent sources include a compound which becomes electronically excited by a chemical reaction and may then emit light which serves as the detectible signal or donates energy to a fluorescent acceptor. A diverse number of families of compounds have been found to provide chemiluminescence under a variety of conditions. One family of compounds is 2,3-dihydro-1,-4-phthalazinedione. The most popular compound is luminol, which is the 5-amino compound. Other members of the family include the 5-amino-6,7,8-trimethoxy- and the dimethylamino›calbenz analog. These compounds can be made to luminesce with alkaline hydrogen peroxide or calcium hypochlorite and base. Another family of compounds is the 2,4,5-triphenylimidazoles, with lophine as the common name for the parent product. Chemiluminescent analogs include para-dimethylamino and -methoxy substituents. Chemiluminescence may also be obtained with oxalates, usually oxalyl active esters, e.g., p-nitrophenyl and a peroxide, e.g., hydrogen peroxide, under basic conditions. Alternatively, luciferins may be used in conjunction with luciferase or lucigenins to provide bioluminescence.

Spin labels are provided by reporter molecules with an unpaired electron spin which can be detected by electron spin resonance (ESR) spectroscopy. Exemplary spin labels include organic free radicals, transitional metal complexes, particularly vanadium, copper, iron, and manganese, and the like. Exemplary spin labels include nitroxide free radicals.

In addition, amplified sequences may be subjected to other post amplification treatments. For example, in some cases, it may be desirable to fragment the sequence prior to hybridization with an oligonucleotide array, in order to provide segments which are more readily accessible to the probes, which avoid looping and/or hybridization to multiple probes. Fragmentation of the nucleic acids may generally be carried out by physical, chemical or enzymatic methods that are known in the art.

Following the various sample preparation operations, the sample will generally be subjected to one or more analysis operations. Particularly preferred analysis operations include, e.g. sequence based analyses using an oligonucleotide array and/or size based analyses using, e.g. microcapillary array electrophoresis.

In some embodiments it may be desirable to provide an additional, or alternative means for analyzing the nucleic acids from the sample

Microcapillary array electrophoresis generally involves the use of a thin capillary or channel which may or may not be filled with a particular separation medium. Electrophoresis of a sample through the capillary provides a size based separation profile for the sample.

Microcapillary array electrophoresis generally provides a rapid method for size based sequencing, PCR product analysis and restriction fragment sizing. The high surface to volume ratio of these capillaries allows for the application of higher electric fields across the capillary without substantial thermal variation across the capillary, consequently allowing for more rapid separations. Furthermore, when combined with confocal imaging methods these methods provide sensitivity in the range of attomoles, which is comparable to the sensitivity of radioactive sequencing methods.

In many capillary electrophoresis methods, the capillaries e.g. fused silica capillaries or channels etched, machined or molded into planar substrates, are filled with an appropriate separation/sieving matrix. Typically, a variety of sieving matrices are known in the art may be used in the microcapillary arrays. Examples of such matrices include, e.g. hydroxyethyl cellulose, polyacrylamide and agarose. Gel matrices may be introduced and polymerized within the capillary channel. However, in some cases this may result in entrapment of bubbles within the channels which can interfere with sample separations. Accordingly, it is often desirable to place a preformed separation matrix within the capillary channel(s), prior to mating the planar elements of the capillary portion. Fixing the two parts, e.g. through sonic welding, permanently fixes the matrix within the channel. Polymerization outside of the channels helps to ensure that no bubbles are formed. Further, the pressure of the welding process helps to ensure a void-free system.

In addition to its use in nucleic acid "fingerprinting" and other sized based analyses the capillary arrays may also be used in sequencing applications. In particular, gel based sequencing techniques may be readily adapted for capillary array electrophoresis.

In addition to detection of mRNA or as the sole detection method expression products from the genes discussed above may be detected as indications of the biological condition of the tissue. Expression products may be detected in either the tissue sample as such, or in a body fluid sample, such as blood, serum, plasma, faeces, mucus, sputum, cerebrospinal fluid, and/or urine of the individual.

The expression products, peptides and proteins, may be detected by any suitable technique known to the person skilled in the art.

In a preferred embodiment the expression products are detected by means of specific antibodies directed to the various expression products, such as immunofluorescent and/or immunohistochemical staining of the tissue.

Immunohistochemical localization of expressed proteins may be carried out by immunostaining of tissue sections from the single tumors to determine which cells expressed the protein encoded by the transcript in question. The transcript levels may be used to select a group of proteins supposed to show variation from sample to sample making a rough correlation between the level of protein detected and the intensity of the transcript on the microarray possible.

For example sections may be cut from paraffin-embedded tissue blocks, mounted, and deparaffinized by incubation at 80 C° for 10 min. followed by immersion in heated oil at 60° C for 10 min. (Estisol 312, Estichem A/S, Denmark) and rehydration. Antigen retrieval is achieved in TEG (TrisEDTA-Glycerol) buffer using microwaves at 900 W. The tissue sections may be cooled in the buffer for 15 min before a brief rinse in tap water. Endogenous peroxidase activity is blocked by incubating the sections with 1% H202 for 20 min. followed by three rinses in tap water, 1 min each. The sections may then be soaked in PBS buffer for 2 min. The next steps can be modified from the descriptions given by Oncogene Science Inc., in the Mouse Immunohistochemistry Detection System, XHCO1 (UniTect, Uniondale, NY, USA). Briefly, the tissue sections are incubated overnight at 4° C with primary antibody (against beta-2 microglobulin (Dako), cytokeratin 8, cystatin-C (both from Europa, US), junB, CD59, E-cadherin, apo-E, cathepsin E, vimentin, IGFII (all from Santa Cruz), followed by three rinses in PBS buffer for 5 min each. Afterwards, the sections are incubated with biotinylated secondary antibody for 30 min, rinsed three times with PBS buffer and subsequently incubated with ABC (avidin-biotinlylated horseradish peroxidase complex) for 30 min. followed by three rinses in PBS buffer.

Staining may be performed by incubation with AEC (3-amino-ethylcarbazole) for 10 min. The tissue sections are counter stained with Mayers hematoxylin, washed in tap water for 5 min. and mounted with glycerol-gelatin. Positive and negative controls may be included in each staining round with all antibodies.

In yet another embodiment the expression products may be detected by means of conventional enzyme assays, such as ELISA methods.

Furthermore, the expression products may be detected by means of peptide/protein chips capable of specifically binding the peptides and/or proteins assessed. Thereby an expression pattern may be obtained.

Assay

In a further aspect the invention relates to an assay for predicting the prognosis of a biological condition in animal tissue, comprising

at least one first marker capable of detecting an expression level of at least one gene selected from the group of genes consisting of gene No. 1 to gene No. 562.

Preferably the assay further comprises means for correlating the expression level to at least one standard expression level and/or at least one reference pattern.

The means for correlating preferably includes one or more standard expression levels and/or reference patterns for use in comparing or correlating the expression levels or patterns obtained from a tumor under examination to the standards.

Preferably the invention relates to an assay for determining an expression pattern of a bladder cell, comprising at least a first marker and/or a second marker, wherein the first marker is capable of detecting a gene from a first gene group as defined above, and/or the second marker is capable of detecting a gene from a second gene group as defined above, correlating the first expression level and/or the second expression level to a standard level of the assessed genes to predict the prognosis of a biological condition in the animal tissue.
The marker(s) are preferably specifically detecting a gene as identified herein.

As described above, it is preferred to determine the expression level from more than one gene, and correspondingly, it is preferred to include more than one marker in the assay, such as at least two markers, such as at least three markers, such as at least four markers, such as at least five markers, such as at least six markers, such as at least seven markers, such as at least eight markers, such as at least nine markers, such as at least ten markers, such as at least 15 markers.

When using markers for at least two different groups, it is preferred that the above number of markers relate to markers in each group.

As discussed above the marker may be any nucleotide probe, such as a DNA, RNA, PNA, or LNA probe capable of hybridising to mRNA indicative of the expression level. The hybridisation conditions are preferably as described below for probes. In another embodiment the marker is an antibody capable of specifically binding the expression product in question.

Patterns can be compared manually by a person or by a computer or other machine. An algorithm can be used to detect similarities and differences. The algorithm may score and compare, for example, the genes which are expressed and the genes which are not

expressed. Alternatively, the algorithm may look for changes in intensity of expression of a particular gene and score changes in intensity between two samples. Similarities may be determined on the basis of genes which are expressed in both samples and genes which are not expressed in both samples or on the basis of genes whose intensity of expression are numerically similar.

Generally, the detection operation will be performed using a reader device external to the diagnostic device. However, it may be desirable in some cases to incorporate the data gathering operation into the diagnostic device itself.

The detection apparatus may be a fluorescence detector, or a spectroscopic detector, or another detector.

Although hybridization is one type of specific interaction which is clearly useful for use in this mapping embodiment antibody reagents may also be very useful.

Gathering data from the various analysis operations, e.g. oligonucleotide and/or microcapillary arrays will typically be carried out using methods known in the art. For example, the arrays may be scanned using lasers to excite fluorescently labeled targets that have hybridized to regions of probe arrays mentioned above, which can then be imaged using charged coupled devices ("CCDs") for a wide field scanning of the array. Alternatively, another particularly useful method for gathering data from the arrays is through the use of laser confocal microscopy which combines the ease and speed of a readily automated process with high resolution detection.

Following the data gathering operation, the data will typically be reported to a data analysis operation. To facilitate the sample analysis operation, the data obtained by the reader from the device will typically be analyzed using a digital computer. Typically, the computer will be appropriately programmed for receipt and storage of the data from the device, as well as for analysis and reporting of the data gathered, i.e., interpreting fluorescence data to determine the sequence of hybridizing probes, normalization of background and single base mismatch hybridizations, ordering of sequence data in SBH applications, and the like.

The invention also relates to a pharmaceutical composition for treating a biological condition, such as bladder tumors.

In one embodiment the pharmaceutical composition comprises one or more of the peptides being expression products as defined above. In a preferred embodiment, the peptides are bound to carriers. The peptides may suitably be coupled to a polymer carrier, for example a

protein carrier, such as BSA. Such formulations are well-known to the person skilled in the art.

The peptides may be suppressor peptides normally lost or decreased in tumor tissue administered in order to stabilise tumors towards a less malignant stage. In another embodiment the peptides are onco-peptides capable of eliciting an immune response towards the tumor cells.

In another embodiment the pharmaceutical composition comprises genetic material, either genetic material for substitution therapy, or for suppressing therapy as discussed below.

In a third embodiment the pharmaceutical composition comprises at least one antibody produced as described above.

In the present context the term pharmaceutical composition is used synonymously with the term medicament. The medicament of the invention comprises an effective amount of one or more of the compounds as defined above, or a composition as defined above in combination with pharmaceutically acceptable additives. Such medicament may suitably be formulated for oral, percutaneous, intramuscular, intravenous, intracranial, intrathecal, intracerebroventricular, intranasal or pulmonal administration. For most indications a localised or substantially localised application is preferred.

Strategies in formulation development of medicaments and compositions based on the compounds of the present invention generally correspond to formulation strategies for any other protein-based drug product. Potential problems and the guidance required to overcome these problems are dealt with in several textbooks, e.g. "Therapeutic Peptides and Protein Formulation. Processing and Delivery Systems", Ed. A.K. Banga, Technomic Publishing AG, Basel, 1995.

Injectables are usually prepared either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. The preparation may also be emulsified. The active ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like, and combinations thereof. In addition, if desired, the preparation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or which enhance the effectiveness or transportation of the preparation.

Formulations of the compounds of the invention can be prepared by techniques known to the

person skilled in the art. The formulations may contain pharmaceutically acceptable carriers and excipients including microspheres, liposomes, microcapsules and nanoparticles.

The preparation may suitably be administered by injection, optionally at the site, where the active ingredient is to exert its effect. Additional formulations which are suitable for other modes of administration include suppositories, and in some cases, oral formulations. For suppositories, traditional binders and carriers include polyalkylene glycols or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient(s) in the range of from 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and generally contain 10-95% of the active ingredient(s), preferably 25-70%.

The preparations are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, e.g. the weight and age of the subject, the disease to be treated and the stage of disease. Suitable dosage ranges are of the order of several hundred µg active ingredient per administration with a preferred range of from about 0.1 µg to 1000 µg, such as in the range of from about 1 µg to 300 µg, and especially in the range of from about 10 µg to 50 µg. Administration may be performed once or may be followed by subsequent administrations. The dosage will also depend on the route of administration and will vary with the age and weight of the subject to be treated. A preferred dosis would be in the interval 30 mg to 70 mg per 70 kg body weight.

Some of the compounds of the present invention are sufficiently active, but for some of the others, the effect will be enhanced if the preparation further comprises pharmaceutically acceptable additives and/or carriers. Such additives and carriers will be known in the art. In some cases, it will be advantageous to include a compound, which promote delivery of the active substance to its target.

In many instances, it will be necessary to administrate the formulation multiple times. Administration may be a continuous infusion, such as intraventricular infusion or administration in more doses such as more times a day, daily, more times a week, weekly, etc.

Vaccines

In a further embodiment the present invention relates to a vaccine for the prophylaxis or treatment of a biological condition comprising at least one expression product from at least one gene said gene being expressed as defined above.

The term vaccines is used with its normal meaning, i.e preparations of immunogenic material for administration to induce in the recipient an immunity to infection or intoxication by a given infecting agent. Vaccines may be administered by intravenous injection or through oral, nasal and/or mucosal administration. Vaccines may be either simple vaccines prepared from one species of expression products, such as proteins or peptides, or a variety of expression products, or they may be mixed vaccines containing two or more simple vaccines. They are prepared in such a manner as not to destroy the immunogenic material, although the methods of preparation vary, depending on the vaccine.

The enhanced immune response achieved according to the invention can be attributable to e.g. an enhanced increase in the level of immunoglobulins or in the level of T-cells including cytotoxic T-cells will result in immunisation of at least 50% of individuals exposed to said immunogenic composition or vaccine, such as at least 55%, for example at least 60%, such as at least 65%, for example at least 70%, for example at least 75%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 92%, such as at least 94%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 98.5%, for example at least 99%, for example at least 99.5% of the individuals exposed to said immunogenic composition or vaccine are immunised.

Compositions according to the invention may also comprise any carrier and/or adjuvant known in the art including functional equivalents thereof. Functionally equivalent carriers are capable of presenting the same immunogenic determinant in essentially the same steric conformation when used under similar conditions. Functionally equivalent adjuvants are capable of providing similar increases in the efficacy of the composition when used under similar conditions.

Therapy

The invention further relates to a method of treating individuals suffering from the biological condition in question, in particular for treating a bladder tumor.

Accordingly, the invention relates to a method for reducing cell tumorigenicity or malignancy of a cell, said method comprising contacting a tumor cell with at least one peptide expressed by at least one gene selected from the group of genes consisting of gene No. 200-214, 233, 234, 235, 236, 244, 249, 251, 252, 255, 256, 259, 261, 262, 266, 268, 269, 273, 274, 275,

276, 277, 279, 280, 281, 282, 285, 286, 289, 293, 295, 296, 299, 301, 304, 306, 307, 308, 311, 312, 313, 314 , 320 , 322, 323, 325, 326, 327, 328 , 330, 331, 332, 333, 334, 338, 341, 342, 343, 345, 348, 349, 350, 351, 352, 353, 355, 357, 360, 361, 363, 366, 367, 370, 373, 374, 375, 376, 385, 386, 387, 389, 390, 392, 394, 398, 400, 401, 405, 406, 407, 408, 410, 411, 412, 414, 415, 416, 418, 424, 426, 428, 433, 434, 435, 436, 438, 439, 440, 441, 442, 443, 445, 446, 453, 460, 461, 463, 464, 465, 466, 467, 469, 470, 471, 472, 473, 475, 476, 477, 479, 480, 481, 482, 483, 485, 486, 487, 488, 490, 492, 494, 496, 497, 498 , 499, 503, 515, 516, 517, 521, 526, 527, 528, 530 ,532, 533, 537, 539, 540, 541, 542, 543, 545, 554, 557, 560.

In order to increase the effect several different peptides may be used simultaneously, such as wherein the tumor cell is contacted with at least two different peptides.

In one embodiment the invention relates to a method of substitution therapy, ie. administration of genetic material generally expressed in normal cells, but lost or decreased in biological condition cells (tumor suppressors). Thus, the invention relates to a method for reducing cell tumorigenicity or malignancy of a cell, said method comprising

obtaining at least one gene selected from the group of genes consisting of gene No. 200-214, 233, 234, 235, 236, 244, 249, 251, 252, 255, 256, 259, 261, 262, 266, 268, 269, 273, 274, 275, 276, 277, 279, 280, 281, 282, 285, 286, 289, 293, 295, 296, 299, 301, 304, 306, 307, 308, 311, 312, 313, 314 , 320 , 322, 323, 325, 326, 327, 328 , 330, 331, 332, 333, 334, 338, 341, 342, 343, 345, 348, 349, 350, 351, 352, 353, 355, 357, 360, 361, 363, 366, 367, 370, 373, 374, 375, 376, 385, 386, 387, 389, 390, 392, 394, 398, 400, 401, 405, 406, 407, 408, 410, 411, 412, 414, 415, 416, 418, 424, 426, 428, 433, 434, 435, 436, 438, 439, 440, 441, 442, 443, 445, 446, 453, 460, 461, 463, 464, 465, 466, 467, 469, 470, 471, 472, 473, 475, 476, 477, 479, 480, 481, 482, 483, 485, 486, 487, 488, 490, 492, 494, 496, 497, 498 , 499, 503, 515, 516, 517, 521, 526, 527, 528, 530 ,532, 533, 537, 539, 540, 541, 542, 543, 545, 554, 557, 560,

introducing said at least one gene into the tumor cell in a manner allowing expression of said gene(s).

In one embodiment at least one gene is introduced into the tumor cell. In another embodiment at least two genes are introduced into the tumor cell.

In one aspect of the invention small molecules that either inhibit increased gene expression or their effects or substitute decreased gene expression or their effects, are introduced to the cellular environment or the cells. Application of small molecules to tumor cells may be

performed by e.g. local application or intravenous injection or by oral ingestion. Small molecules have the ability to restore function of reduced gene expression in tumor or cancer tissue.

In another aspect the invention relates to a therapy whereby genes (increase and/or decrease) generally are correlated to disease are inhibited by one or more of the following methods:

A method for reducing cell tumorigenicity or malignancy of a cell, said method comprising

obtaining at least one nucleotide probe capable of hybridising with at least one gene of a tumor cell, said at least one gene being selected from the group of genes consisting of gene Nos. 1-199, 215-232, 237, 238, 239, 240, 241, 242, 243, 245, 246, 247, 248, 250, 253, 254, 257, 258, 260, 263, 264, 265, 267, 270, 271, 272, 278, 283, 284, 287, 288, 290, 291, 292, 294, 297, 298, 300, 302, 303, 305, 309, 310, 315, 316, 317, 318, 319, 321, 324, 329, 335, 336, 337, 339, 340, 344, 346, 347, 354, 356, 358, 359, 362, 364, 365, 368, 369, 371, 372, 377, 378, 379, 380, 381, 382, 383, 384, 388, 391, 393, 395, 396, 397, 399, 402, 403, 404, 409, 413, 417, 419, 420, 421, 422, 423, 425, 427 ,429, 430, 431, 432, 437, 444, 447, 448, 449, 450, 451, 452, 454, 455 ,456, 457, 458, 459, 462, 468, 474, 478, 484, 489, 491, 493, 495, 500, 501, 502, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 518 , 519, 520, 522, 523, 524, 525, 529, 531, 534, 535, 536, 538, 544, 546, 547, 548, 549, 550, 551, 552, 553, 555, 556, 558, 559, 561, 562,

introducing said at least one nucleotide probe into the tumor cell in a manner allowing the probe to hybridise to the at least one gene, thereby inhibiting expression of said at least one gene. This method is preferably based on anti-sense technology, whereby the hybridisation of said probe to the gene leads to a down-regulation of said gene.

In another preferred embodiment, the method for reducing cell tumorigenicity or malignancy of a cell is based on RNA interference, comprising small interfering RNAs (siRNAs) specifically directed against at least one gene being selected from the group of genes consisting of gene Nos. 1-199, 215-232, 237, 238, 239, 240, 241, 242, 243, 245, 246, 247, 248, 250, 253, 254, 257, 258, 260, 263, 264, 265, 267, 270, 271, 272, 278, 283, 284, 287, 288, 290, 291, 292, 294, 297, 298, 300, 302, 303, 305, 309, 310, 315, 316, 317, 318, 319, 321, 324, 329, 335, 336, 337, 339, 340, 344, 346, 347, 354, 356, 358, 359, 362, 364, 365, 368, 369, 371, 372, 377, 378, 379, 380, 381, 382, 383, 384, 388, 391, 393, 395, 396, 397, 399, 402, 403, 404, 409, 413, 417, 419, 420, 421, 422, 423, 425, 427 ,429, 430, 431, 432, 437, 444, 447, 448, 449, 450, 451, 452, 454, 455 ,456, 457, 458, 459, 462, 468, 474, 478, 484, 489, 491, 493, 495, 500, 501, 502, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513,

514, 518 , 519, 520, 522, 523, 524, 525, 529, 531, 534, 535, 536, 538, 544, 546, 547, 548, 549, 550, 551, 552, 553, 555, 556, 558, 559, 561, 562.

The down-regulation may of course also be based on a probe capable of hybridising to regulatory components of the genes in question, such as promoters.

The hybridization may be tested in vitro at conditions corresponding to in vivo conditions. Typically, hybridization conditions are of low to moderate stringency. These conditions favour specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the lower the salt concentration and the higher the temperature the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional consideration is whether one of the nucleic acids is immobilized (for example on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a deter-

gent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

In another aspect a method of reducing tumoregeneicity relates to the use of antibodies against an expression product of a cell from the biological tissue. The antibodies may be produced by any suitable method, such as a method comprising the steps of

obtaining expression product(s) from at least one gene said gene being expressed as defined above,

immunising a mammal with said expression product(s) obtaining antibodies against the expression product.

Use

The methods described above may be used for producing an assay for diagnosing a biological condition in animal tissue, or for identification of the origin of a piece of tissue. Further, the methods of the invention may be used for prediction of a disease course and treatment response.

Furthermore, the invention relates to the use of a peptide as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

Furthermore, the invention relates to the use of a gene as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

Also, the invention relates to the use of a probe as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

The genetic material discussed above for may be any of the described genes or functional parts thereof. The constructs may be introduced as a single DNA molecule encoding all of the genes, or different DNA molecules having one or more genes. The constructs may be introduced simultaneously or consecutively, each with the same or different markers.

The gene may be linked to the complex as such or protected by any suitable system normally used for transfection such as viral vectors or artificial viral envelope, liposomes or micellas, wherein the system is linked to the complex.

Numerous techniques for introducing DNA into eukaryotic cells are known to the skilled artisan. Often this is done by means of vectors, and often in the form of nucleic acid encapsidated by a (frequently virus-like) proteinaceous coat. Gene delivery systems may be applied to a wide range of clinical as well as experimental applications.

Vectors containing useful elements such as selectable and/or amplifiable markers, promoter/enhancer elements for expression in mammalian, particularly human, cells, and which may be used to prepare stocks of construct DNAs and for carrying out transfections are well known in the art. Many are commercially available.

Various techniques have been developed for modification of target tissue and cells in vivo. A number of virus vectors, discussed below, are known which allow transfection and random integration of the virus into the host. See, for example, Dubensky et al. (1984) Proc. Natl. Acad. Sci. USA 81:7529-7533; Kaneda et al., (1989) Science 243:375-378; Hiebert et al. (1989) Proc. Natl. Acad. Sci. USA 86:3594-3598; Hatzoglu et al., (1990) J. Biol. Chem. 265:17285-17293; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381. Routes and modes of administering the vector include injection, e.g intravascularly or intramuscularly, inhalation, or other parenteral administration.

Advantages of adenovirus vectors for human gene therapy include the fact that recombination is rare, no human malignancies are known to be associated with such viruses, the adenovirus genome is double stranded DNA which can be manipulated to accept foreign genes of up to 7.5 kb in size, and live adenovirus is a safe human vaccine organisms.

Another vector which can express the DNA molecule of the present invention, and is useful in gene therapy, particularly in humans, is vaccinia virus, which can be rendered non-replicating (U.S. Pat. Nos. 5,225,336; 5,204,243; 5,155,020; 4,769,330).

Based on the concept of viral mimicry, artificial viral envelopes (AVE) are designed based on the structure and composition of a viral membrane, such as HIV-1 or RSV and used to deliver genes into cells in vitro and in vivo. See, for example, U.S. Pat. No. 5,252,348, Schreier H. et al., J. Mol. Recognit., 1995, 8:59-62; Schreier H et al., J. Biol. Chem., 1994, 269:9090-9098; Schreier, H., Pharm. Acta Helv. 1994, 68:145-159; Chander, R et al. Life Sci., 1992, 50:481-489, which references are hereby incorporated by reference in their entirety. The envelope is preferably produced in a two-step dialysis procedure where the "naked" enve-

lope is formed initially, followed by unidirectional insertion of the viral surface glycoprotein of interest. This process and the physical characteristics of the resulting AVE are described in detail by Chander et al., (supra). Examples of AVE systems are (a) an AVE containing the HIV-1 surface glycoprotein gp160 (Chander et al., supra; Schreier et al., 1995, supra) or glycosyl phosphatidylinositol (GPI)-linked gp120 (Schreier et al., 1994, supra), respectively, and (b) an AVE containing the respiratory syncytial virus (RSV) attachment (G) and fusion (F) glycoproteins (Stecenko, A. A. et al., Pharm. Pharmacol. Lett. 1:127-129 (1992)). Thus, vesicles are constructed which mimic the natural membranes of enveloped viruses in their ability to bind to and deliver materials to cells bearing corresponding surface receptors.

AVEs are used to deliver genes both by intravenous injection and by instillation in the lungs. For example, AVEs are manufactured to mimic RSV, exhibiting the RSV F surface glycoprotein which provides selective entry into epithelial cells. F-AVE are loaded with a plasmid coding for the gene of interest, (or a reporter gene such as CAT not present in mammalian tissue).

The AVE system described herein in physically and chemically essentially identical to the natural virus yet is entirely "artificial", as it is constructed from phospholipids, cholesterol, and recombinant viral surface glycoproteins. Hence, there is no carry-over of viral genetic information and no danger of inadvertant viral infection. Construction of the AVEs in two independent steps allows for bulk production of the plain lipid envelopes which, in a separate second step, can then be marked with the desired viral glycoprotein, also allowing for the preparation of protein cocktail formulations if desired.

Another delivery vehicle for use in the present invention are based on the recent description of attenuated Shigella as a DNA delivery system (Sizemore, D. R. et al., Science 270:299-302 (1995), which reference is incorporated by reference in its entirety). This approach exploits the ability of Shigellae to enter epithelial cells and escape the phagocytic vacuole as a method for delivering the gene construct into the cytoplasm of the target cell. Invasion with as few as one to five bacteria can result in expression of the foreign plasmid DNA delivered by these bacteria.

A preferred type of mediator of nonviral transfection in vitro and in vivo is cationic (ammonium derivatized) lipids. These positively charged lipids form complexes with negatively charged DNA, resulting in DNA charged neutralization and compaction. The complexes endocytosed upon association with the cell membrane, and the DNA somehow escapes the endosome, gaining access to the cytoplasm. Cationic lipid:DNA complexes appear highly stable under normal conditions. Studies of the cationic lipid DOTAP suggest the complex dissociates when the inner layer of the cell membrane is destabilized and anionic lipids from

the inner layer displace DNA from the cationic lipid. Several cationic lipids are available commercially. Two of these, DMRI and DC-cholesterol, have been used in human clinical trials. First generation cationic lipids are less efficient than viral vectors. For delivery to lung, any inflammatory responses accompanying the liposome administration are reduced by changing the delivery mode to aerosol administration which distributes the dose more evenly.

Drug screening

Genes identified as changing in various stages of bladder cancer can be used as markers for drug screening. Thus by treating bladder cancer cells with test compounds or extracts, and monitoring the expression of genes identified as changing in the progression of bladder cancers, one can identify compounds or extracts which change expression of genes to a pattern which is of an earlier stage or even of normal bladder mucosa.

It is also within the scope of the invention to use small molecules in drug screening.

The following are non-limiting examples illustrating the present invention.

EXAMPLES

Example 1

Identification of a molecular signature defining disease progression in patients with superficial bladder carcinoma

Patient samples

Bladder tumor biopsies were obtained directly from surgery after removal of the necessary amount of tissue for routine pathology examination. The tumors were frozen at -80°C in a guanidinium thiocyanate solution for preservation of the RNA. Informed consent was obtained in all cases, and the protocols were approved by the scientific ethical committee of Aarhus County. The samples for the no progression group were selected by the following criteria: a) Ta or T1 tumors with no prior higher stage tumors; b) a minimum follow up period of 12 months to the most recent routine cystoscopy examination of the bladder with no occurrence of tumors of higher stage. The samples for the progression group were selected by two criteria: a) Ta or T1 tumors with no prior higher stage tumors; b) subsequent progression to a higher stage tumor, see Table 1.

Table 1. Clinical data on all patients involved in the study

Training set

Group	Sample	Hist.	Progressed to:	Time to progression	Follow-up time months
No prog.	150-6	Ta gr3	-	-	44
No prog.	997-1	Ta gr2	-	-	24
No prog.	833-2	Ta gr3	-	-	35
No prog.	1070-1	Ta gr3	-	-	33
No prog.	968-1	Ta gr2	-	-	26
No prog.	625-1	T1 gr3	-	-	12
No prog.	880-1	T1 gr3	-	-	47
No prog.	815-1	Ta gr2	-	-	49
No prog.	861-1	Ta gr2	-	-	45
No prog.	669-1	Ta gr2	-	-	55
No prog.	368-4	Ta gr2	-	-	16
No prog.	898-1	Ta gr2	-	-	17
No prog.	576-6	Ta gr2	-	-	36
Prog.	747-3	Ta gr2	T1 gr3	6	
Prog.	956-2	Ta gr3	T1 gr3	27	-
Prog.	1083-1	Ta gr2	T1 gr3	1	-
Prog.	686-3	Ta gr2	T1 gr2	6	-
Prog.	795-13	Ta gr2	T1 gr3	4	-
Prog.	865-1	Ta gr2	T1 gr2	5	-
Prog.	112-2	Ta gr3	T1 gr3	7	-
Prog.	825-3	Ta gr3	T1 gr3	6	-
Prog.	679-2	Ta gr2	T2+ gr3	31	-
Prog.	941-4	Ta gr3	T2+ gr3	10	-
Prog.	607-1	T1 gr2	T2+ gr3	3	-
Prog.	1017-1	T1 gr3	T2+ gr3	8	-
Prog.	1276-1	T1 gr3	T2+ gr3	7	-
Prog.	501-1	T1 gr3	T2+ gr3	26	-
Prog.	744-1	T1 gr3	T2+ gr3	14	-
Prog.	839-1	T1 gr3	T2+ gr3	12	-

Test set

Group	Sample	Hist.	Progressed to:	Time to progression	Follow-up time months
No prog.	1008-1	Ta gr2	-	-	55
No prog.	1060-1	Ta gr2	-	-	48
No prog.	1086-1	Ta gr2	-	-	34
No prog.	1105-1	Ta gr2	-	-	31
No prog.	1145-1	Ta gr2	-	-	39
No prog.	1352-1	Ta gr2	-	-	26

No prog.	829-1	Ta gr2	-	-	37
No prog.	942-1	Ta gr2	-	-	37
No prog.	780-1	Ta gr2	-	-	50
Prog	1327-1	Ta gr2	T1 gr3	8	-
Prog.	1062-2	Ta gr3	T1 gr3	4	-
Prog.	1354-1	Ta gr3	T1 gr3	8	-
Prog.	1093-1	Ta gr3	T1 gr3	5	-
Prog.	925-7	Ta gr2	T1 gr3	4	-
Prog.	962-10	Ta gr0	T2+ gr3	1	-
Prog.	970-1	Ta gr3	T2+ gr3	1	-
Prog.	1027-1	Ta gr3	T2+ gr3	2	-
Prog.	1252-1	T1 gr3	T2+ gr3	5	-
Prog.	1191-1	T1 gr4	T2+ gr4	1	-

Delineation of non-progressing tumors from progressing tumors

To delineate non-progressing tumors from progressing tumors we now profiled a total of 29 bladder tumor samples; 13 early stage bladder tumor samples without progression (median follow-up time 35 months) and 16 early stage bladder tumor samples with progression (median time to progression 7 months). See Table 1 for description of patient disease courses. We analyzed gene expression changes between the two groups of tumors by hybridizing the labeled RNA samples to customized Affymetrix GeneChips with 59,000 probe-sets to cover virtually the entire transcriptome (~95% coverage). Low expressed and non-varying probe-sets were eliminated from the data set and the resulting 6,647 probe-sets that showed variation across the tumor samples were subjected to further analysis. These probe-sets represent 5,356 unique genes (Unigene clusters).

Gene expression similarities between tumor biopsies

We analyzed gene expression similarities between the tumor biopsies using unsupervised hierarchical cluster analysis (Fig. 1). This showed a notable distinction between the non-progressing and the progressing tumors when using the 3,197 most varying probe-sets (s.d. ≥ 75) for clustering (4 errors; χ^2 test, $P = 0.0001$). Using other gene-sets based on different gene variation criteria demonstrated the same distinction between the tumor groups. Two of the samples that show later progression (825-3 and 112-2) were found in the non-progression branch of the cluster dendrogram and two of the non-progressing samples (815-1 and 150-6) were found in the progression branch. This distinct separation of the samples indicated a considerable biological difference between the two groups of tumors. Notably, the T1 tumors did not cluster separately from Ta tumors; however, they did form a sub-cluster in the progressing branch of the dendrogram. Based on this we decided to look for a general signature of progression disregarding pathologic staging of the tumors.

Selection of the 100 most significantly up-regulated genes in each group using t-test statistics

We delineated the non-progressing tumors from the progressing tumors by selecting the 100 most significantly up-regulated genes in each group using t-test statistics (Fig. 2 and Table 2). Among the genes up regulated in the non-progressing group we found the *SERPINB5* and *FAT* tumor suppressor genes and the *FGFR3* gene, which has been shown to be frequently mutated in superficial bladder tumors with low recurrence rates (van Rhijn et al. 2001). Among the genes up regulated in the progressing group we found the *PLK* (Yuan et al. 1997), *CDC25B* (Galaktionov et al. 1991), *CDC20* (Weinstein et al. 1994) and *MCM7* (Hiraiwa et al. 1997) genes, which are involved in regulating cell cycle and cell proliferation. Furthermore, in this group we identified the *WHSC1*, *DD96* and *GRB7* genes, which have been predicted/computed (Gene Ontology) to be involved in oncogenic transformation. Another interesting candidate in this group is the *NRG1* gene, which through interaction with the HER2/HER3 receptors has been found to induce differentiation of lung epithelial cells (Liu & Kern 2002). The *PPARD* gene was also identified as up regulated in the tumors that show later progression. Disruption of this gene was found to decrease tumorigenicity in colon cancer cells (Park et al. 2001). Furthermore, *PPARD* regulates *VEGF* expression in bladder cancer cell lines (Fauconnet et al. 2002).

Table 2. The 200 best markers of progression

Eos Hu03 ID	Unigene Build 133	Description	T-test	5% perm	Exemplar accession#
416640	Hs.79404	neuron-specific protein	6.03	5.62	BE262478
442220	Hs.8148	selenoprotein T	5.98	5.06	AL037800
426982	Hs.173091	ubiquitin-like 3	5.9	4.88	AA149707
416815	Hs.80120	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)	5.52	4.67	U41514
435521	Hs.6361	mitogen-activated protein kinase kinase 1 interacting protein 1	5.24	4.51	W23814
447343	Hs.236894	ESTs, Highly similar to S02392 alpha-2-macroglobulin receptor precursor [H.sapiens]	5.23	4.44	AA256641
452829	Hs.63368	ESTs, Weakly similar to TRHY_HUMAN TRICHOHYALI [H.sapiens]	4.95	4.39	AI955579
414895	Hs.116278	Homo sapiens cDNA FLJ13571 fis, clone PLACE1008405	4.94	4.31	AW894856
426252	Hs.28917	ESTs	4.9	4.26	BE176980
444604	Hs.11441	chromosome 1 open reading frame 8	4.89	4.17	AW327695
409632	Hs.55279	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	4.89	4.13	W74001
446556	Hs.15303	KIAA0349 protein	4.87	4.08	AB002347
426799	Hs.303154	popeye protein 3	4.86	4.03	H14843
428115	Hs.300855	KIAA0977 protein	4.86	4.00	AB023194
419847	Hs.184544	Homo sapiens, clone IMAGE:3355383, mRNA, partial cds	4.82	3.97	AW390601
417839	Hs.82712	fragile X mental retardation, autosomal homolog 1	4.8	3.93	AI815732

428284	Hs.183435	NM_004545:Homo sapiens NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 1 (7kD, MNLL) (NDUFB1), mRNA.	4.78	3.92	AA535762
422929	Hs.94011	ESTs, Weakly similar to MGB4_HUMAN MELANOMA-ASSOCIATED ANTIGEN B4 [H.sapiens]	4.77	3.90	AA356694
414762	Hs.77257	KIAA0068 protein	4.72	3.86	AW068349
453395	Hs.377915	mannosidase, alpha, class 2A, member 1	4.71	3.84	D63998
421311	Hs.283609	hypothetical protein PRO2032	4.65	3.82	N71848
446847	Hs.82845	Homo sapiens cDNA: FLJ21930 fis, clone HEP04301, highly similar to HSU90916 Human clone 23815 mRNA sequence	4.65	3.82	T51454
413840	Hs.356228	RNA binding motif protein, X chromosome	4.62	3.79	AI301558
418321	Hs.84087	KIAA0143 protein	4.62	3.78	D63477
430604	Hs.247309	succinate-CoA ligase, GDP-forming, beta subunit	4.61	3.74	AV650537
423185	Hs.380062	ornithine decarboxylase antizyme 1	4.61	3.74	BE299590
417615	Hs.82314	hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	4.6	3.70	BE548641
418504	Hs.85335	Homo sapiens mRNA; cDNA DKFZp564D1462 (from clone DKFZp564D1462)	4.59	3.68	BE159718
400846	-	sortilin-related receptor, L(DLR class) A repeats-containing (SORL1)	4.57	3.66	-
426028	Hs.172028	a disintegrin and metalloproteinase domain 10 (ADAM10)	4.53	3.65	NM_00111 0
425243	Hs.155291	KIAA0005 gene product	4.47	3.63	N89487
434978	Hs.4310	eukaryotic translation initiation factor 1A	4.45	3.62	AA321238
409513	Hs.54642	methionine adenosyltransferase II, beta	4.43	3.59	AW966728
433282	Hs.49007	hypothetical protein	4.43	3.56	BE539101
421628	Hs.106210	hypothetical protein FLJ10813	4.37	3.56	AL121317
452170	Hs.28285	patched related protein translocated in renal cancer	4.37	3.54	AF064801
440014	Hs.6856	ash2 (absent, small, or homeotic, Drosophila, homolog)-like	4.37	3.52	AW960782
431857	Hs.271742	ADP-ribosyltransferase (NAD; poly (ADP-ribose) poly-merase)-like 3	4.36	3.52	W19144
417924	Hs.82932	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	4.35	3.51	AU077231
421733	Hs.1420	fibroblast growth factor receptor 3 (achondroplasia, thanato-phoric dwarfism)	4.34	3.50	AL119671
440197	Hs.317714	pallid (mouse) homolog, pallidin	4.32	3.49	AW340708
434055	Hs.3726	x 003 protein	4.32	3.48	AF168712
445831	Hs.13351	LanC (bacterial lantibiotic synthetase component C)-like 1	4.31	3.46	NM_00605 5
439632	Hs.334437	hypothetical protein MGC4248	4.29	3.45	AW410714
448813	Hs.22142	cytochrome b5 reductase b5R.2	4.28	3.44	AF169802
449268	Hs.23412	hypothetical protein FLJ20160	4.28	3.43	AW369278
429311	Hs.198998	conserved helix-loop-helix ubiquitous kinase	4.28	3.42	AF080157
423599	Hs.31731	peroxiredoxin 5	4.27	3.41	AI805664
422913	Hs.121599	CGI-18 protein	4.26	3.40	NM_01594 7
418127	Hs.83532	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	4.26	3.39	BE243982

425221	Hs.155188	TATA box binding protein (TBP)-associated factor, RNA polymerase II, F, 55kD	4.25	3.38	AV649864
426682	Hs.2056	UDP glycosyltransferase 1 family, polypeptide A9	4.23	3.37	AV660038
421101	Hs.101840	major histocompatibility complex, class I-like sequence	4.23	3.37	AF010446
444037	Hs.380932	CHMP1.5 protein	4.22	3.35	AV647686
443407	Hs.348514	ESTs, Moderately similar to 2109260A B cell growth factor [H.sapiens]	4.21	3.35	AA037683
448625	Hs.178470	hypothetical protein FLJ22662	4.21	3.34	AW970786
450997	Hs.35254	hypothetical protein FLB6421	4.16	3.34	AW580830
444336	Hs.10882	HMG-box containing protein 1	4.15	3.33	AF019214
416977	Hs.406103	hypothetical protein FKSG44	4.14	3.32	AW130242
420613	Hs.406637	ESTs, Weakly similar to A47582 B-cell growth factor precur-sor [H.sapiens]	4.13	3.31	AI873871
414843	Hs.77492	heterogeneous nuclear ribonucleoprotein A0	4.1	3.30	BE386038
408288	Hs.16886	gb:zl73d06.r1 Stratagene colon (937204) Homo sapiens cDNA clone 5', mRNA sequence	4.09	3.29	AA053601
422043	Hs.110953	retinoic acid induced 1	4.09	3.29	AL133649
432864	Hs.359682	calpastatin	4.08	3.28	D16217
410047	Hs.379753	zinc finger protein 36 (KOX 18)	4.06	3.28	AI167810
400773	-	NM_003105*:Homo sapiens sortilin-related receptor, L(DLR class) A repeats-containing (SORL1), mRNA.	4.06	3.27	-
423960	Hs.136309	SH3-containing protein SH3GLB1	4.05	3.27	AA164516
449626	Hs.112860	zinc finger protein 258	4.04	3.27	AA774247
429953	Hs.226581	COX15 (yeast) homolog, cytochrome c oxidase assembly protein	4.04	3.24	NM_00437 6
428901	Hs.146668	KIAA1253 protein	4.02	3.24	AI929568
420079	Hs.94896	PTD011 protein	3.99	3.22	NM_01405 1
436576	Hs.77542	ESTs	3.98	3.21	AI458213
412841	Hs.101395	hypothetical protein MGC11352	3.97	3.21	AI751157
431604	Hs.264190	vacuolar protein sorting 35 (yeast homolog)	3.96	3.21	AF175265
428318	Hs.356190	ubiquitin B	3.96	3.19	BE300110
430677	Hs.359784	desmoglein 2	3.95	3.19	Z26317
407955	Hs.9343	ESTs	3.94	3.18	BE536739
426177	Hs.167700	Homo sapiens cDNA FLJ10174 fis, clone HEMBA1003959	3.92	3.17	AA373452
429802	Hs.5367	ESTs, Weakly similar to I38022 hypothetical protein [H.sapiens]	3.92	3.17	H09548
423810	Hs.132955	BCL2/adenovirus E1B 19kD-interacting protein 3-like	3.92	3.16	AL132665
421475	Hs.104640	HIV-1 inducer of short transcripts binding protein; lymphoma related factor	3.91	3.15	AF000561
436472	Hs.46366	KIAA0948 protein	3.91	3.14	AL045404
434263	Hs.79187	ESTs	3.9	3.13	N34895
400843	-	NM_003105*:Homo sapiens sortilin-related receptor, L(DLR class) A repeats-containing (SORL1), mRNA.	3.9	3.13	-
440357	Hs.20950	phospholysine phosphohistidine inorganic pyrophosphate phosphatase	3.89	3.12	AA379353
437223	Hs.330716	Homo sapiens cDNA FLJ14368 fis, clone HEMBA1001122	3.88	3.12	C15105

426125	Hs.166994	FAT tumor suppressor (Drosophila) homolog	3.86	3.11	X87241
432554	Hs.278411	NCK-associated protein 1	3.86	3.10	AI479813
422506	Hs.300741	sorcin	3.85	3.10	R20909
413786	Hs.13500	ESTs	3.83	3.09	AW613780
429561	Hs.250646	baculoviral IAP repeat-containing 6	3.83	3.08	AF265555
404977	-	Insulin-like growth factor 2 (somatomedin A) (IGF2)	3.83	3.08	-
427722	Hs.180479	hypothetical protein FLJ20116	3.82	3.08	AK000123
400844	-	NM_003105*:Homo sapiens sortilin-related receptor, L(DLR class) A repeats-containing (SORL1), mRNA.	3.82	3.08	-
426469	Hs.363039	methylmalonate-semialdehyde dehydrogenase	3.81	3.07	BE297886
439578	Hs.350547	nuclear receptor co-repressor/HDAC3 complex subunit	3.81	3.06	AW263124
426508	Hs.170171	glutamate-ammonia ligase (glutamine synthase)	3.8	3.06	W23184
448524	Hs.21356	hypothetical protein DKFZp762K2015	3.79	3.06	AB032948
448357	Hs.108923	RAB38, member RAS oncogene family	3.79	3.06	N20169
425097	Hs.154545	PDZ domain containing guanine nucleotide exchange factor(GEF)1	3.77	3.05	NM_014247
421649	Hs.106415	peroxisome proliferative activated receptor, delta	5.76	5.50	AA721217
427747	Hs.180655	serine/threonine kinase 12	5.41	5.03	AW411425
439010	Hs.75216	Homo sapiens cDNA FLJ13713 fis, clone PLACE2000398, moderately similar to LAR PROTEIN PRECURSOR (LEUKOCYTE ANTIGEN RELATED) (EC 3.1.3.48)	4.57	4.80	AW170332
438818	Hs.30738	ESTs	4.49	4.59	AW979008
438013	Hs.15670	ESTs	4.42	4.50	AI002106
452929	Hs.172816	neuregulin 1	4.37	4.40	AW954938
404826	-	Target Exon	4.22	4.32	-
429124	Hs.196914	minor histocompatibility antigen HA-1	4.2	4.26	AW505086
421505	Hs.285641	KIAA1111 protein	4.16	4.24	AW249934
428712	Hs.190452	KIAA0365 gene product	4.14	4.19	AW085131
427239	Hs.356512	ubiquitin carrier protein	4.11	4.10	BE270447
421595	Hs.301685	KIAA0620 protein	4.1	4.07	AB014520
433844	Hs.179647	Homo sapiens cDNA FLJ12195 fis, clone MAMMA1000865	4.04	4.02	AA610175
443679	Hs.9670	hypothetical protein FLJ10948	4.01	4.00	AK001810
422959	Hs.349256	paired immunoglobulin-like receptor beta	4.01	3.98	AV647015
452012	Hs.279766	kinesin family member 4A	3.98	3.96	AA307703
435320	Hs.117864	ESTs	3.97	3.91	AA677934
456332	Hs.399939	gb:nc39d05.r1 NCI_CGAP_Pr2 Homo sapiens cDNA clone, mRNA sequence	3.95	3.88	AA228357
427999	Hs.181369	ubiquitin fusion degradation 1-like	3.94	3.86	AI435128
427681	Hs.284232	tumor necrosis factor receptor superfamily, member 12 (translocating chain-association membrane protein)	3.93	3.81	AB018263
413929	Hs.75617	collagen, type IV, alpha 2	3.93	3.79	BE501689
420116	Hs.95231	FH1/FH2 domain-containing protein	3.9	3.77	NM_013241
433914	Hs.112160	Homo sapiens DNA helicase homolog (PIF1) mRNA, partial cds	3.88	3.75	AF108138
420732	Hs.367762	ESTs	3.87	3.74	AA789133
452517	-	gb:RC-BT068-130399-068 BT068 Homo sapiens cDNA,	3.84	3.70	AI904891

		mRNA sequence			
437524	Hs.385719	ESTs	3.82	3.68	AI627565
435158	Hs.65588	DAZ associated protein 1	3.8	3.66	AW663317
448780	Hs.267749	Human DNA sequence from clone 366N23 on chromosome 6q27. Contains two genes similar to consecutive parts of the C. elegans UNC-93 (protein 1, C46F11.1) gene, a KIAA0173 and Tubulin-Tyrosine Ligase LIKE gene, a Mitotic Feedback Control Protein MADP2 H	3.8	3.65	W92071
445084	Hs.250848	hypothetical protein FLJ14761	3.79	3.64	H38914
423138	-	gb:EST385571 MAGE resequences, MAGM Homo sapiens cDNA, mRNA sequence	3.75	3.60	AW973426
419602	Hs.91521	hypothetical protein	3.74	3.59	AW248434
442549	Hs.8375	TNF receptor-associated factor 4	3.74	3.58	AI751601
450893	Hs.25625	hypothetical protein FLJ11323	3.73	3.55	AK002185
414223	Hs.238246	hypothetical protein FLJ22479	3.73	3.55	AA954566
444312	Hs.351142	ESTs	3.72	3.53	R44007
425205	Hs.155106	receptor (calcitonin) activity modifying protein 2	3.71	3.51	NM_005854
432327	Hs.274363	neuroglobin	3.71	3.49	R36571
451970	Hs.211046	ESTs	3.67	3.48	AI825732
408049	Hs.345588	desmoplakin (DPI, DPII)	3.67	3.45	AW076098
440100	Hs.158549	ESTs, Weakly similar to T2D3_HUMAN TRANSCRIPTION INITIATION FACTOR TFIID 135 KDA SUBUNIT [H.sapiens]	3.66	3.45	BE382685
426468	Hs.117558	ESTs	3.65	3.43	AA379306
402384	-	NM_007181*:Homo sapiens mitogen-activated protein kinase kinase kinase kinase 1 (MAP4K1), mRNA.	3.64	3.43	-
458132	Hs.103267	hypothetical protein FLJ22548 similar to gene trap PAT 12	3.64	3.42	AW247012
447400	Hs.18457	hypothetical protein FLJ20315	3.64	3.42	AK000322
443893	Hs.115472	ESTs, Weakly similar to 2004399A chromosomal protein [H.sapiens]	3.63	3.41	BE079602
424959	Hs.153937	activated p21cdc42Hs kinase	3.62	3.40	NM_005781
409586	Hs.55044	DKFZP586H2123 protein	3.6	3.39	AL050214
445692	Hs.182099	ESTs	3.6	3.37	AI248322
433052	Hs.293003	ESTs, Weakly similar to PC4259 ferritin associated protein [H.sapiens]	3.6	3.36	AW971983
421782	Hs.108258	actin binding protein; macrophin (microfilament and actin filament cross-linker protein)	3.59	3.35	AB029290
414907	Hs.77597	polo (Drosophia)-like kinase	3.58	3.34	X90725
454639	-	gb:RC2-ST0158-091099-011-d05 ST0158 Homo sapiens cDNA, mRNA sequence	3.57	3.33	AW811633
434547	Hs.106124	ESTs	3.56	3.32	R26240
439130	Hs.375195	ESTs	3.55	3.32	AA306090
413564	-	gb:601146990F1 NIH_MGC_19 Homo sapiens cDNA clone 5', mRNA sequence	3.54	3.31	BE260120
443471	Hs.398102	Homo sapiens clone FLB3442 PRO0872 mRNA, complete cds	3.53	3.31	AW236939

424415	Hs.146580	enolase 2, (gamma, neuronal)	3.52	3.30	NM_001975
405036	-	NM_021628*:Homo sapiens arachidonate lipoxygenase 3 (ALOXE3), mRNA. VERSION NM_020229.1 GI	3.52	3.29	-
422068	Hs.104520	Homo sapiens cDNA FLJ13694 fis, clone PLACE2000115	3.52	3.29	AI807519
424244	Hs.143601	hypothetical protein hCLA-iso	3.52	3.28	AV647184
451867	Hs.27192	hypothetical protein dJ1057B20.2	3.51	3.26	W74157
429187	Hs.163872	ESTs, Weakly similar to S65657 alpha-1C-adrenergic receptor splice form 2 [H.sapiens]	3.49	3.26	AA447648
415200	Hs.78202	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	3.48	3.25	AL040328
405667	-	Target Exon	3.48	3.25	-
421075	Hs.101474	KIAA0807 protein	3.47	3.23	AB018350
424909	Hs.153752	cell division cycle 25B	3.46	3.22	S78187
451164	Hs.60659	ESTs, Weakly similar to T46471 hypothetical protein DKFZp434L0130.1 [H.sapiens]	3.46	3.21	AA015912
438644	Hs.129037	ESTs	3.46	3.20	AI126162
432258	Hs.293039	ESTs	3.45	3.19	AW973078
411817	Hs.72241	mitogen-activated protein kinase kinase 2	3.45	3.19	BE302900
414918	Hs.72222	hypothetical protein FLJ13459	3.45	3.18	AI219207
437256	Hs.97871	Homo sapiens, clone IMAGE:3845253, mRNA, partial cds	3.43	3.17	AL137404
404208	-	C6001282:gi|4504223|ref|NP_000172.1| glucuronidase, beta [Homo sapiens] gi|114963|sp|P082	3.42	3.16	-
421989	Hs.110457	Wolf-Hirschhorn syndrome candidate 1	3.4	3.15	AJ007042
438942	Hs.6451	PRO0659 protein	3.39	3.14	AW875398
412649	Hs.74369	integrin, alpha 7	3.38	3.14	NM_002206
414840	Hs.23823	hairy/enhancer-of-split related with YRPW motif-like	3.37	3.13	R27319
434831	Hs.273397	KIAA0710 gene product	3.35	3.12	AA248060
431842	Hs.271473	epithelial protein up-regulated in carcinoma, membrane associated protein 17	3.34	3.11	NM_005764
402328	-	Target Exon	3.34	3.10	-
405371	-	NM_005569*:Homo sapiens LIM domain kinase 2 (LIMK2), transcript variant 2a, mRNA.	3.33	3.10	-
441650	Hs.132545	ESTs	3.32	3.09	AI261960
418629	Hs.86859	growth factor receptor-bound protein 7	3.3	3.09	BE247550
406002	-	Target Exon	3.3	3.08	-
420307	Hs.66219	ESTs	3.29	3.08	AW502869
425093	Hs.154525	KIAA1076 protein	3.28	3.07	AB028999
427351	Hs.123253	hypothetical protein FLJ22009	3.28	3.07	AW402593
417900	Hs.82906	CDC20 (cell division cycle 20, S. cerevisiae, homolog)	3.28	3.06	BE250127
457228	Hs.195471	Human cosmid CRI-JC2015 at D10S289 in 10sp13	3.27	3.05	U15177
421026	Hs.101067	GCN5 (general control of amino-acid synthesis, yeast, homolog)-like 2	3.27	3.04	AL047332
430746	Hs.406256	ESTs	3.27	3.03	AW977370
409556	Hs.54941	phosphorylase kinase, alpha 2 (liver)	3.27	3.03	D38616
451225	Hs.57655	ESTs	3.26	3.03	AI433694

404913	-	NM_024408*:Homo sapiens Notch (Drosophila) homolog 2 (NOTCH2), mRNA. VERSION NM_024410.1 GI	3.25	3.02	-
404875	-	NM_022819*:Homo sapiens phospholipase A2, group IIF (PLA2G2F), mRNA. VERSION NM_020245.2 GI	3.23	3.02	-
404606	-	Target Exon	3.23	3.01	-
414732	Hs.77152	minichromosome maintenance deficient (S. cerevisiae) 7	3.22	3.01	AW410976
425380	Hs.32148	AD-015 protein	3.22	3.00	AA356389
421186	Hs.270563	ESTs, Moderately similar to T12512 hypothetical protein DKFZp434G232.1 [H.sapiens]	3.21	2.98	AI798039
445462	Hs.288649	hypothetical protein MGC3077	3.2	2.97	AA378776

Permutation analysis of 100 most significantly up-regulated genes in each group

By permuting the sample labels 500 times we estimated the significance of the differentially expressed genes. The permutation analysis revealed that it was highly unlikely to find as good markers by chance, as similar godd markers were only found in 5% of the permutated data sets, see Table 2.

Molecular predictor of progression

A molecular predictor of progression using a combination of genes may have higher prediction accuracy than when using single marker genes. Therefore, to identify the gene-set that gives the best prediction results using the lowest number of genes we built a predictor using the "leave one out" cross-validation approach, as previously described (Golub et al. 1999). Selecting the 100 best genes in each cross-validation loop gave the lowest number of prediction errors (5 errors, 83% correct classification) in our training set consisting of the 29 tumors (see Figure 3). As in our previous study we used a maximum likelihood classification approach. We selected a gene-expression signature consisting of those 45 genes that were present in 75% of the cross-validation loops, and these represent our optimal gene-set for progression prediction (Fig. 4*a* and Table 3).

Many of these 45 genes were also found among the 200 best markers of progression, however, the cross-validation approach also identified other interesting markers of progression like *BIRC5* (Survivin), an apoptosis inhibitor that is up regulated in the tumors that show later progression. *BIRC5* has been reported to be expressed in most common cancers (Ambrosini et al. 1997). To validate the significance of the 45-gene expression signature we used a test set consisting of 19 early stage bladder tumors (9 tumors with no progression and 10 tumors with later progression). Total RNA from these samples were amplified, labeled and hybridized to customized 60mer-oligonucleotide microarray glass slides and the relative expressions of the 45 classifier genes were measured following appropriate normalization and background adjustments of the microarray data. The independent tumor samples were clas-

sified as non-progressing or progressing according to the degree of correlation to the average no progression profile from the training samples (Fig. 3*b*). When applying no cutoff limits to the predictions the predictor identified 74% of the samples correctly. However, as done recently in a breast cancer study (van't Veer et al. 2002), we applied correlation cutoff limits of 0.1 and –0.1 in order to disregard samples with really low correlation values and in this way we obtained 92% correct predictions of samples with correlation values above 0.1 or below –0.1. Although the test-set is limited in size the performance is notable and could be of clinical use.

Table 3. The 45 optimal genes for disease progression prediction.

Eos Hu03 ID	Unigene Build 133	Description	T-Test	5% perm	Gene Name	Exemplar Accession	CV
439010	Hs.75216	protein tyrosine phosphatase, receptor type, F	4.57	4.39	PTPRF	AW170332	29
429124	Hs.196914	minor histocompatibility antigen HA-1	4.20	4.09	*HA-1*	AW505086	29
421649	Hs.106415	peroxisome proliferative activated receptor, delta	5.76	5.64	*PPARD*	AA721217	29
433914	Hs.112160	DNA helicase homolog (PIF1)	3.88	3.61	*PIF1*	AF108138	29
429187	Hs.163872	ESTs, Weakly similar to hypothetical protein FLJ20489	3.49	3.17	-	AA447648	28
422765	Hs.1578	baculoviral IAP repeat-containing 5 (survivin)	2.68	2.56	*BIRC5*	AW409701	28
433844	Hs.179647	ESTs	4.04	3.80		AA610175	26
450893	Hs.25625	Hypothetical protein FLJ11323	3.73	3.46	*FLJ11323*	AK002185	25
452866	Hs.268016	ESTs	3.10	3.02		R26969	24
424909	Hs.153752	cell division cycle 25B	3.46	3.16	*CDC25B*	S78187	24
452929	Hs.172816	neuregulin 1	4.37	4.23	*NRG1*	AW954938	23
420116	Hs.95231	formin homology 2 domain containing 1	3.90	3.63	*FHOD1*	NM_013241	22
453963	Hs.28959	cDNA FLJ36513 fis, clone TRACH2001523	3.44	2.88	-	AA040311	29
429561	Hs.250646	baculoviral IAP repeat-containing 6 (apollon)	3.83	3.03	*BIRC6*	AF265555	29
418127	Hs.83532	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	4.26	3.37	*MCP*	BE243982	29
422119	Hs.111862	KIAA0590 gene product	2.33	1.95	*KIAA0590*	AI277829	29
435521	Hs.6361	mitogen-activated protein kinase kinase 1 interacting protein 1	5.24	4.53	*MAP2K1IP1*	W23814	29
409632	Hs.55279	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	4.89	4.11	*SERPINB5*	W74001	29
452829	Hs.63368	ESTs	4.95	4.31	-	AI955579	29
416640	Hs.79404	DNA segment on chromosome 4 (unique) 234 expressed sequence	6.03	5.51	*D4S234E*	BE262478	29
425097	Hs.154545	PDZ domain containing guanine nucleotide exchange factor(GEF)1	3.77	3.18	*PDZ-GEF1*	NM_014247	28

445926	Hs.334826	splicing factor 3b, subunit 1, 155kDa	2.40	2.03	*SF3B1*	AF054284	28
437325	Hs.5548	F-box and leucine-rich repeat protein 5	2.48	2.09	*FBXL5*	AF142481	28
448813	Hs.22142	cytochrome b5 reductase b5R.2	4.28	3.41	*LOC51700*	AF169802	28
426799	Hs.303154	ESTs	4.86	4.04	-	H14843	28
446847	Hs.82845	ESTs	4.65	3.79	-	T51454	28
428016	Hs.181461	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila)	3.77	3.15	*ARIH1*	AJ243190	27
418321	Hs.84087	KIAA0143 protein	4.62	3.76	*KIAA0143*	D63477	27
422984	Hs.351597	ESTs	3.50	2.93		W28614	26
408688	Hs.152925	KIAA1268 protein	3.52	2.95	*KIAA1268*	AI634522	26
440357	Hs.20950	phospholysine phosphohistidine inorganic pyrophosphate phosphatase	3.89	3.07	*LHPP*	AA379353	26
420269	Hs.96264	alpha thalassemia/mental retardation syndrome X-linked (RAD54 (S. cerevisiae) homolog)	3.39	2.85	*ATRX*	U72937	26
423185	?	ornithine decarboxylase antizyme 1	4.61	3.71	OAZ1	BE299590	26
443407	Hs.348514	clone IMAGE:4052238, mRNA, partial cds	4.21	3.32	-	AA037683	25
457329	Hs.359682	calpastatin	3.59	2.99	*CAST*	AI634860	25
452714	Hs.30340	KIAA1165: likely ortholog of mouse Nedd4 WW domain-binding protein 5A	3.62	3.01	*KIAA1165*	AW770994	25
444773	Hs.11923	hypothetical protein DJ167A19.1	3.71	3.11	*DJ167A19.1*	BE156256	24
418504	Hs.85335	ESTs	4.59	3.67	-	BE159718	24
444604	Hs.11441	Chromosome 1 open reading frame 8	4.89	4.17	*C1orf8*	AW327695	23
410691	Hs.65450	reticulon 4			*RTN4*	AW239226	23
430604	Hs.247309	succinate-CoA ligase, GDP-forming, beta subunit	4.61	3.72	*SUCLG2*	AV650537	23
421311	Hs.283609	muscleblind-like protein MBLL39	4.65	3.82	*MBLL39*	N71848	23
439632	Hs.334437	hypothetical protein MGC4248	4.29	3.42	*MGC4248*	AW410714	22
417924	Hs.82932	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	4.35	3.49	*CCND1*	AU077231	22
453395	Hs.377915	mannosidase, alpha, class 2A, member 1	4.71	3.84	*MAN2A1*	D63998	22

Permutation analysis of 45 genes

Again permutation analysis revealed that for all of the 45 genes similar good markers were only found in 5% of the 500 permuted datasets (see Table 3).

Expression profiling of metachrone higher stage tumors

Expression profiling of the metachrone higher stage tumors could provide important information on the degree of expression similarities between the primary and the secondary tumors. Tissues from secondary tumors were available from 14 of the patients with disease progression and these were also hybridized to the customized Affymetrix GeneChips.

Hierarchical cluster analysis of all tumor samples based on the 3,213 most varying probesets showed that tumors originating from the same patient in 9 of the cases clustered tightly together indicating a high degree of intra individual similarity in expression profiles (Fig. 5). Notable, one tight clustering pair of tumors was a Ta and a T2+ tumor (patient 941). It was remarkable that Ta and T1 tumors and T1 or T2+ tumors from a single individual were more similar than e.g. Ta tumors from two individuals. There was no correlation between presence and absence of the tight clustering of samples from the same patient and time interval to tumor progression. The tight clustering of the 9 tumor pairs probably reflects the monoclonal nature of many bladder tumors (Sidransky et al. 1997). A set of genomic abnormalities like chromosomal gains and losses characterize bladder tumors of different stages from single individuals (Primdahl et al. 2002), and such physical abnormalities could be one of the causes of the strong similarity of metachronous tumors. The fact that 5 of the tumor pairs clustered apart may be explained by an oligoclonal origin of these tumors.

Customized GeneChip design, normalization and expression measures

We used a customized Affymetrix GeneChip (Eos Hu03) designed by Eos Biotech Inc., as described (Eaves et al. 2002). Approximately 45,000 mRNA/EST clusters and 6,200 predicted exons are represented by the 59,000 probesets on Eos Hu03 array. Data were normalized using protocols and software developed at Eos Biotechnology, Inc. (WO0079465). An "average intensity" (AI) for each probeset was calculated by taking the trimean of probe intensities following background subtraction and normalization to a gamma distribution (Turkey 1977).

cRNA preparation, array hybridization and scanning

Preparation of cRNA from total RNA and subsequent hybridization and scanning of the customized GeneChip microarrays (Eos Hu03) were performed as described previousley (Dyrskjot et al. 2003).

Custom oligonucleotide microarray procedures

Three 60mer oligonucleotides were designed for each of the 45 genes using Array Designer 2.0. All steps in the customized oligonucleotide microarray analysis were performed essentially as described (Kruhoffer et al.) Each of the probes was spotted in duplicates and all hybridisations were carried out twice. The samples were labelled with Cy3 and a common reference pool was labelled with Cy5. The reference pool was made by pooling of cRNA generated from investigated samples and from universal human RNA. Following scanning of the glass slides the fluorescent intensities were quantified and background adjusted using SPOT 2.0 (Jain et al. 2002). Data were subsequently normalized using a LOWESS normalisation procedure implemented in the SMA package to R. To select the best oligonucleotide probe for each of the 45 genes, 13 of the samples from the training set were re-analysed on

the custom oligonucleotide microarray platform and the obtained expression ratios were compared to the expression levels from the Affymetrix GeneChips. The oligonucleotide probes with the highest correlation to the Affymetrix GeneChip probes were selected.

Expression data analysis

Before analysing the expression data from the Eos Hu03 GeneChips control probes were removed and only probes with AI levels above 100 in at least 8 experiments and with max/min equal to or above 1.6 were selected. This filtering generated a gene-set consisting of 6,647 probes for further analysis. Average linkage hierarchical cluster analysis of the tumour samples was carried out using a modified Pearson correlation as similarity metric (Eisen et al. 1998). Genes and arrays were median centered and normalised to the magnitude of 1 before clustering. We used the GeneCluster 2.0 software for the supervised selection of markers and for performing permutation tests. The 45 genes for predicting progression were selected by t-test statistics and cross-validation performance as previously described (Dyrskjot et al. 2003) and independent samples were classified according to the correlation to the average no progression signature profile of the 45 genes.

EXAMPLE 2

Identifying distinct classes of bladder carcinoma using microarrays

Patient disease course information – class discovery

We selected tumours from the entire spectrum of bladder carcinoma for expression profiling in order to discover the molecular classes of the disease. The tumours analysed are listed in Table 4 below together with the available patient disease course information.

Table 4 Disease course information of all patients involved- class discovery.

Group	Patient	Previous tumours	Tumour examined on array	Pattern	Reviewed histology	Subsequent tumours	Carcinoma *in situ**
A	709-1		Ta gr 2 (200297)	Papillary	Ta gr3		no
	968-1		Ta gr 2 (011098)	Papillary	+	Ta gr 2 (150101)	no
	934-1		Ta gr 2 (220798)	Papillary	+		no
	928-1		Ta gr 2 (240698)	Papillary	+		no
	930-1		Ta gr 2 (300698)	Papillary	+		no
B	989-1		Ta gr 3 (281098)	Papillary	+		no
	1264-1		Ta gr 3 (130600)	Papillary	+	Ta gr 2 (231000) Ta gr 2 (220101) Ta gr 2 (300401)	no
	876-5	Ta gr 2 (230398) Ta gr 2 (271098) Ta gr 2 (090699) Ta gr 2 (011199)	Ta gr 3 (170400)	Papillary	+		no

	669-7	Ta gr 2 (101296) Ta gr 2 (150897) Ta gr 1 (161297) Ta gr 3 (270498) Ta gr 2 (220299)	Ta gr 3 (230899)	Papillary	Ta gr2	Ta gr 2 (120100) Ta gr 2 (250500) Ta gr 2 (250900) Ta gr 2 (050201)	no
	716-2	Ta gr 2 (070397)	Ta gr 3 (230497)	Papillary	+	Ta gr 2 (040697) Ta gr 1 (170698)	no
C	1070-1		Ta gr 3 (150399)	Papillary	+	Ta gr 3 (291099)	Subsequent visit
	956-2		Ta gr 3 (061299)	Papillary	+	Ta gr 3 (061200)	Sampling visit
	1062-2		Ta gr 3 (120799)	Papillary	+	T1 gr 3 (161199)	Sampling visit
	1166-1		Ta gr 3 (271099)	Papillary	+		Sampling visit
	1330-1		Ta gr 3 (311000)	Papillary	+		Sampling visit
D	112-10	Ta gr 2 (070794) Ta gr 3 (011294) T1 gr 3(150695) Ta gr 3 (121095) T1 gr 3(040396) Ta gr 2 (200896) Ta gr 2 (111296) Ta gr 2 (230497) Ta gr 2 (030997)	Ta gr 3 (060198)	Papillary	+	Ta gr 3 (110698) T1 gr 3 (191098) Ta gr 3 (240299) T1 gr 3 (050799) T1 gr 3 (081199) T1 gr 3 (180400)	Previous visit
	320-7	T1 gr 3 (011194) T1 gr 3 (150896) Ta gr 3 (100897)	Ta gr 3 (290997)	Papillary	+	Ta gr 3 (290198) Ta gr 3 (290698)	Sampling visit
	747-7	Ta gr 2 (010597) Ta gr 2 (220597) Ta gr 2 (230997) Ta gr 2 (260198) T1 gr 3 (270498) Ta gr 2 (170898)	Ta gr 3 (161298)	Papillary	+	Ta gr 2 (050599) Ta gr 2 (280999) Ta gr 2 (141299)	Sampling visit
	967-3	T1 gr 3 (280998) T1 gr 3 (250199)	Ta gr 3 (140699)	Papillary	+	T1 gr 3 (080999)	Sampling visit
E	625-1		T1 gr 3 (200996)	Papillary	+		No
	847-1		T1 gr 3 (210198)	Papillary	+		No
	1257-1		T1 gr 3 (240500)	Solid	+		Sampling visit
	919-1		T1 gr 3 (220698)	Papillary	+		No
	880-1		T1 gr 3 (300398)	Papillary	+	Ta gr 2 (091198) Ta gr 1 (090399) Ta gr 2 (050900) Ta gr 2 (190301)	No
	812-1		T1 gr 3 (061098)	Papillary	+		No
	1269-1		T1 gr 3 (230600)	Papillary	-		No
	1083-2	Ta gr 2 (280499)	T1 gr 3 (120599)	Papillary	-		No
	1238-1		T1 gr 3 (020500)	Papillary	+	T2 gr 3 (211100) Ta gr 2 (211100)	No
	1065-1		T1 gr 3 (160399)	Papillary	-		Subsequent visit
	1134-1		T1 gr 3 (181099)	Papillary	T2 gr3	T1 gr 3 (280200)	Sampling visit

						T1 gr 3 (020500) T1 gr 3 (131100)	
F	1164-1		T2+ gr 4 (101299)	Solid	gr 3		No
	1032-1		T2+ gr ? (050199)	Mixed	-		Not measured
	1117-1		T2+ gr 3 (010999)	Solid	+		Sampling visit
	1178-1		T2+ gr 3 (200100)	Solid	+		Not measured
	1078-1		T2+ gr 3 (120499)	Solid	+		Not measured
	875-1		T2+ gr 3 (180398)	Solid	+		No
	1044-1		T2+ gr 3 (010299)	Solid	+	T2+ gr 3 (060999)	Not measured
	1133-1		T2+ gr 3 (081099)	Solid	+		Not measured
	1068-1		T2+ gr 3 (220399)	Solid	+		No
	937-1		T2+ gr 3 (280798)	Solid	-		Not measured

Group A: Ta gr2 tumours – no recurrence within 2 years.

Group B: Ta gr3 tumours – no prior T1 tumour and no carcinoma *in situ* in random biopsies.

Group C: Ta gr3 tumours – no prior T1 tumour but carcinoma *in situ* in random biopsies.

Group D: Ta gr3 tumours – a prior T1 tumour and carcinoma *in situ* in random biopsies.

Group E: T1 gr3 tumours – no prior T2+ tumour. Group F: T2+ tumours gr3/4 – only primary tumours.

* Carcinoma *in situ* detected in selected site biopsies at previous, sampling or subsequent visits.

Two-way hierarchical cluster analysis of tumor samples

A two-way hierarchical cluster analysis of the tumour samples based on the 1767 gene-set (see class discovery using hierachical clustering) remarkably separated all 40 tumours according to conventional pathological stages and grades with only few exceptions (Fig. 6a). We identified two main branches containing the superficial Ta tumours, and the invasive T1 and T2+ tumours. In the superficial branch two sub-clusters of tumours could be identified, one holding 8 tumours that had frequent recurrences and one holding 3 out of the five Ta grade 2 tumours with no recurrences. In the invasive branch, it was notable that four Ta grade 3 tumours clustered tightly with the muscle invasive T2+ tumours. These four Ta tumours, from patients with no previous tumour history, showed concomitant CIS in the surrounding mucosa, indicating that this sub-fraction of Ta tumours has some of the more aggressive features found in muscle invasive tumours. The stage T1 cluster could be separated into three sub-clusters with no clear clinical difference. The one stage T1 grade 3 tumour that clustered with the stage T2+ muscle invasive tumours was the only T1 tumour that showed a solid growth pattern, all others showing papillary growth. Nine out of ten T2+ tumours were found in one single cluster. The remarkable distinct separation of the tumour groups according to stage, with practically no overlap between groups, was also demonstrated by multidimensional scaling analysis (Fig. 6c).

In an attempt to reduce the number of genes needed for class prediction we identified those genes that were scored by the Cancer Genome Anatomy Project (at NCI) as belonging to cancer-related groups such as tumour suppressors, oncogenes, cell cycle, etc. These genes were then selected from the initial 1767 gene-set, and those 88 which showed largest variation (SD of the gene vector >=4), were used for hierarchical clustering of the tumour samples. The obtained clusters was almost identical to the 1767 gene-set cluster dendrogram (Fig. 6b), indicating that the tumour clustering does not simply reflect larger amounts of stromal components in the invasive tumour biopsies.

The clustering of the 1767 genes revealed several characteristic profiles in which there was a distinct difference between the tumour groups (Fig. 6d; black lines identifying clusters a to j).

Cluster a, shows a high expression level in all the Ta grade 3 tumours (Fig. 7a) and, as a novel finding, contains genes encoding 8 transcription factors as well as other nuclear genes related to transcriptional activity. Cluster c contains genes that are up-regulated in both Ta grade 3 with high recurrence rate and CIS, in T2+ and some T1 tumours. This cluster shows a remarkable tight co-regulation of genes related to cell cycle control and mitosis (Fig. 7c). Genes encoding cyclins, PCNA as well as a number of centromere related proteins are present in this cluster. They indicate increased cellular proliferation and may form new targets for small molecule therapy (Seymour 1999). Cluster f shows a tight cluster of genes related to keratinisation (Fig. 7f). Two tumours (875-1 and 1178-1) had a very high expression of these genes and a re-evaluation of the pathology slides revealed that these were the only two samples to show squamous metaplasia. Thus, activation of this cluster of genes promotes the squamous metaplasia not infrequently seen by light microscopy in invasive bladder tumours. The genes in this cluster is listed in Table 5.

Table 5 Genes for classifying samples with squamous metaplasia

Chip acc. #	UniGene Build 162	description
D83657_at	Hs.19413	NM_005621; S100 calcium-binding protein A12
HG3945-HT4215_at		
J00124_at		
L05187_at		
L05188_f_at	Hs.505327	
L10343_at	Hs.112341	NM_002638; skin-derived protease inhibitor 3 preproprotein
L42583_f_at	Hs.367762	NM_005554; keratin 6A
L42601_f_at	Hs.367762	NM_005554; keratin 6A
L42611_f_at	Hs.446417	NM_173086; keratin 6 isoform K6e
M19888_at	Hs.1076	NM_003125; small proline-rich protein 1B (cornifin)
M20030_f_at	Hs.505352	
M21005_at		

M21302_at	Hs.505327	
M21539_at	Hs.2421	NM_006518; small proline-rich protein 2C
M86757_s_at	Hs.112408	NM_002963; S100 calcium-binding protein A7
S72493_s_at	Hs.432448	NM_005557; keratin 16
U70981_at	Hs.336046	NM_000640; interleukin 13 receptor, alpha 2 precursor
V01516_f_at	Hs.367762	NM_005554; keratin 6A
X53065_f_at		
X57766_at	Hs.143751	NM_005940; matrix metalloproteinase 11 preproprotein
Z19574_ma1_at		

Cluster g contains genes that are up-regulated in T2+ tumours and in the Ta grade 3 tumours with CIS that cluster in the invasive branch (Fig. 7g). This cluster contains genes related to angiogenesis and connective tissue such as laminin, myosin, caldesmon, collagen, dystrophin, fibronectin, and endoglin. The increased transcription of these genes may indicate a profound remodelling of the stroma that could reflect signalling from the tumour cells, from infiltrating lymphocytes, or both. Some of these may also form new drug targets (Fox et al. 2001). It is remarkable that these genes are those that most clearly separate the Ta grade 3 tumours surrounded by CIS from all other Ta grade 3 tumours. The presence of adjacent CIS is usually diagnosed by taking a set of eight biopsies from different places in the bladder mucosa. However, the present data clearly indicate that analysis of stroma remodelling genes in the Ta tumours could eliminate this invasive procedure.

The clusters b, d, e, h, i, and j contain genes related to nuclear proteins, cell adhesion, growth factors, stromal proteins, immune system, and proteases, respectively (see Figure 8). A summary of the stage related gene expression is shown in Table 6.

Table 6

Table 6• Summary of stage related gene expression

Functional gene clusters[a]

Tumour stage	Transcription	Nuclear processes	Proliferation	Matrix re-modelling	Extracellular matrix	Immune system
Ta gr2	↑	-	-	-	↓↓	↓
Ta gr3	↑↑↑	↑↑	↑↑	-	↓↓	↓
T1 gr3	↑[b]	-	↑↑[b]	-	↓	↑[b]
T2 gr3	↑	-	↑↑↑	↑↑↑	↑	↑
Ta gr3 + CIS	↑↑↑	↑↑	↑↑↑	↑↑↑	↑	↑

a For a detailed description of gene clusters see Fig. 8.

b An increase in gene expression was only found in about half of the samples analysed.

Class prediction of bladder tumours

An objective class prediction of bladder tumours based on a limited gene-set is clinically usefull. We therefore built a classifier using tumours correctly separated in the three main groups as identified in the cluster dendrogram (Fig. 6a). We used a maximum likelihood classification method with a "leave one out" cross-validation scheme (Shipp et al. 2002; van't Veer et al. 2002) in which one test tumour was removed from the set, and a set of predictive genes was selected from the remaining tumour samples for classifying the test tumour. This process was repeated for all tumours. Predictive genes that showed the largest possible separation of the three groups were selected for classification, and each tumour was classified according to how close it was to the mean of the three groups (Fig. 8a).

Classification of samples

From the hierarchical cluster analysis of the samples (class discovery) we identified three major "molecular classes" of bladder carcinoma highly associated with the pathologic staging of the samples. Based on this finding we decided to build a molecular classifier that assigns tumours to these three "molecular classes". To build the classifier, we only used the tumours in which there was a correlation between the "molecular class" and the associated pathologic stage. Consequently, a T1 tumour clustering in the "molecular class" of T2 tumours was not used to build the classifier.

The genes used in the classifier were those genes with the highest values of the ratio (B/W) of the variation between the groups to the variation within the groups. High values of the ratio (B/W) signify genes with good group separation performance. We calculated the sum over the genes of the squared distance from the sample value to the group mean and classified the sample as belonging to the group where the distance to the group mean was smallest. If the relative difference between the distance to the closest and the second closest group compared to the distance to the closest group were below 5%, the classification failed and the sample was classified as belonging to both groups. The relative difference is refered to as the classifier strength.

Classifier performance

The classifier performance was tested using from 1-160 genes in cross-validation loops. Figure 9 shows that the closest correlation to histopathology is obtained in the cross-validation model using from 69-97 genes. Based on this we chose the model using 80 genes for cross-validation as our final classifier model.

Classifier model using 71 genes

We selected those genes for our final classifier model that were used in at least 75% (25 times) of the cross-validation loops. These 71 genes are listed in table 7.

Table 7 Feature: Accession number on HuGene fl array. Number: Number of times used in the 80 genes cross validation loops. Test (B/W): see below.

Feature	Unigene Build 162	Description	Number	Test (B/W)
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A	33	26.77
D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasciclin I-like)	33	27.71
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor	31	25.78
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor	33	31.18
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor	33	28.29
D89077_at	Hs.75367	NM_006748; Src-like-adaptor	33	30.03
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2	33	51.50
HG4069-HT4339_s_at			27	25.06
HG67-HT67_f_at			33	27.81
HG907-HT907_at			33	25.76
J02871_s_at	Hs.436317	NM_000779; cytochrome P450, family 4, subfamily B, polypeptide 1	33	32.61
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor	33	28.02
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide	33	29.46
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase	33	38.21
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein	33	35.34
J05448_at	Hs.79402	NM_002694; DNA directed RNA polymerase II polypeptide C NM_032940; DNA directed RNA polymerase II polypeptide C	32	26.51
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	33	28.66
L13720_at	Hs.437710	NM_000820; growth arrest-specific 6	33	29.69
M12125_at	Hs.300772	NM_003289; tropomyosin 2 (beta)	28	24.89
M15395_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor	33	29.40
M16591_s_at	Hs.89555	NM_002110; hemopoietic cell kinase isoform p61HCK	33	32.34
M20530_at			33	30.28
M23178_s_at	Hs.73817	NM_002983; chemokine (C-C motif) ligand 3	33	35.36
M32011_at	Hs.949	NM_000433; neutrophil cytosolic factor 2	33	41.88
M33195_at	Hs.433300	NM_004106; Fc fragment of IgE, high affinity I, receptor for, gamma polypeptide precursor	33	30.40
M55998_s_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein	33	26.83
M57731_s_at	Hs.75765	NM_002089; chemokine (C-X-C motif) ligand 2	33	31.84
M68840_at	Hs.183109	NM_000240; monoamine oxidase A	33	32.39
M69203_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor	33	36.21
M72885_ma1_s_at			33	27.94

M83822_at	Hs.209846	NM_006726; LPS-responsive vesicle trafficking, beach and anchor containing	33	26.44
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)	33	49.85
U01833_at	Hs.81469	NM_002484; nucleotide binding protein 1 (MinD homolog, E. coli)	33	30.62
U07231_at	Hs.309763	NM_002092; G-rich RNA sequence binding factor 1	33	39.10
U09937_rna1_s_at			33	30.88
U10550_at	Hs.79022	NM_005261; GTP-binding mitogen-induced T-cell protein NM_181702; GTP-binding mitogen-induced T-cell protein	28	25.26
U20158_at	Hs.2488	NM_005565; lymphocyte cytosolic protein 2	33	32.41
U41315_rna1_s_at			33	43.56
U47414_at	Hs.13291	NM_004354; cyclin G2	33	44.42
U49352_at	Hs.414754	NM_001359; 2,4-dienoyl CoA reductase 1 precursor	33	37.04
U50708_at	Hs.1265	NM_000056; branched chain keto acid dehydrogenase E1, beta polypeptide precursor NM_183050; branched chain keto acid dehydrogenase E1, beta polypeptide precursor	33	42.89
U52101_at	Hs.9999	NM_001425; epithelial membrane protein 3	33	29.86
U64520_at	Hs.66708	NM_004781; vesicle-associated membrane protein 3 (cellubrevin)	33	30.17
U65093_at	Hs.82071	NM_006079; Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	33	32.07
U68019_at	Hs.288261	NM_005902; MAD, mothers against decapentaplegic homolog 3	31	26.70
U68385_at	Hs.380923		33	31.56
U74324_at	Hs.90875	NM_002871; RAB-interacting factor	33	30.26
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;	33	50.37
U90549_at	Hs.236774	NM_006353; high mobility group nucleosomal binding domain 4	33	32.16
X04085_rna1_at			28	25.13
X07743_at	Hs.77436	NM_002664; pleckstrin	33	28.13
X13334_at	Hs.75627	NM_000591; CD14 antigen precursor	33	35.79
X14046_at	Hs.153053	NM_001774; CD37 antigen	30	24.70
X15880_at	Hs.415997	NM_001848; collagen, type VI, alpha 1 precursor	33	31.51
X15882_at	Hs.420269	NM_001849; alpha 2 type VI collagen isoform 2C2 precursor NM_058174; alpha 2 type VI collagen isoform 2C2a precursor NM_058175; alpha 2 type VI collagen isoform 2C2a precursor	33	32.32
X51408_at	Hs.380138	NM_001822; chimerin (chimaerin) 1	33	30.51
X53800_s_at	Hs.89690	NM_002090; chemokine (C-X-C motif) ligand 3	33	33.63
X54489_rna1_at			33	33.57
X57579_s_at			33	41.43
X64072_s_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor	33	43.21
X67491_f_at	Hs.355697	NM_005271; glutamate dehydrogenase 1	33	30.97
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b	33	46.53
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7	33	53.16
X78520_at	Hs.372528	NM_001829; chloride channel 3	33	47.38

Y00787_s_at	Hs.624	NM_000584; interleukin 8 precursor	32	27.54
Z12173_at	Hs.334534	NM_002076; glucosamine (N-acetyl)-6-sulfatase precursor	30	25.44
Z19554_s_at	Hs.435800	NM_003380; vimentin	27	24.59
Z26491_s_at	Hs.240013	NM_000754; catechol-O-methyltransferase isoform MB-COMT NM_007310; catechol-O-methyltransferase isoform S-COMT	32	26.92
Z29331_at	Hs.372758	NM_003344; ubiquitin-conjugating enzyme E2H isoform 1 NM_182697; ubiquitin-conjugating enzyme E2H isoform 2	33	33.49
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865; NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1	33	44.45
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein	33	55.18

Test for significance of classifier

To test the class separation performance of the 71 selected genes we compared the B/W ratios with the similar ratios of all the genes calculated from permutations of the arrays. For each permutation we construct three pseudogroups, pseudo-Ta, pseudo-T1, and pseudo-T2, so that the proportion of samples from the three original groups is approximately the same in the three pseudogroups. We then calculate the ratio of the variation between the psudogroups to the variation within the pseudogroups for all the genes. For 500 permutations we only two times had one gene for which the B/W value was higher than the lowest value for the original B/W values of the 71 selected genes (the two values being 25.28 and 25.93).

The classifier performance was tested using from 1-160 genes in cross-validation loops, and a model using an 80 gene cross-validation scheme showed the best correlation to pathologic staging ($p<10^{-9}$). The 71 genes that were used in at least 75% of the cross validation loops were selected to constitute our final classifier model. See the expression profiles of the 71 genes in Figure 10. The genes are clustered to obtain a better overview of similar expression patterns. From this it is obvious that the T1 stage is characterised by having expression patterns in common with either Ta or T2 tumours. There are no single genes that can be used as a T1 marker.

Permutation analysis

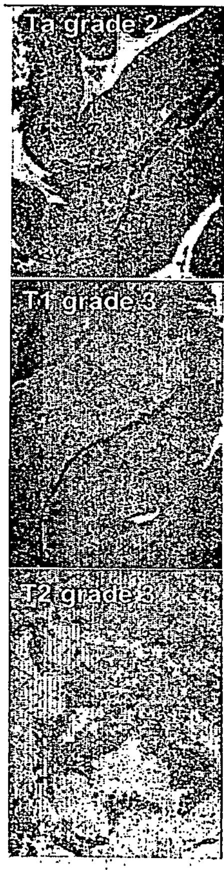
To test the class separation performance of the 71 selected genes we compared their performance to those of a permutated set of pseudo-Ta, T1 and T2 tumours. In 500 permutations we only detected two genes with a performance equal to the poorest performing classifying genes.

Classification using 80 predictive genes and other gene-sets

The classification using 80 predictive genes in cross-validation loops identified the Ta group with no surrounding CIS and no previous tumor or no previous tumor of a higher stage (Table 8). Interestingly, the Ta tumours surrounded by CIS that were classified as T2 or T1 clearly demonstrate the potential of the classification method for identifying surrounding CIS in a non-invasive way, thereby supplementing clinical and pathologic information.

Table 8

Table 8 • Clinical data on disease courses and results of molecular classification



Tumours[a]	Patient	Previous tumours	Tumour analysed	Subsequent tumours	Carcinoma *In situ*[b]	Reviewed histology[c]	Molecular classifier[c] 320	80	20
	Ta grade II tumours – no progression								
	709-1		Ta gr2		No	Ta gr3	Ta	Ta	Ta
	968-1		Ta gr2	1 Ta	No		*Ta/T1*	Ta	Ta
	934-1		Ta gr2		No		*T1*	Ta	Ta
	928-1		Ta gr2		No		Ta	*Ta*	*T1*
	930-1		Ta gr2		No		Ta	Ta	Ta
	Ta grade III tumours – no prior T1 tumour or CIS								
	989-1		Ta gr3		No		Ta	Ta	Ta
	1264-1		Ta gr3	3 Ta	No		Ta	Ta	Ta
	876-5	4 Ta	Ta gr3		No		Ta	Ta	Ta
	669-7	5 Ta	Ta gr3	4 Ta	No	Ta gr2	Ta	Ta	Ta
	716-2	1 Ta	Ta gr3	2 Ta	No		Ta	Ta	Ta
	Ta grade III tumours – no prior T1 tumour but CIS in selected site biopsies								
	1070-1		Ta gr3	1 Ta	Subsequent visit		Ta	Ta	Ta
	956-2		Ta gr3	1 Ta	Sampling visit		T2	T2	T2/T1
	1062-2		Ta gr3	1 T1	Sampling visit		T2/Ta	*T1/Ta*	*Ta*
	1166-1		Ta gr3		Sampling visit		*Ta/T1*	Ta	Ta
	1330-1		Ta gr3		Sampling visit		T2	T2	Ta
	Ta grade III tumours – a prior T1 tumour and CIS in selected site biopsies								
	747-7	5 Ta, 1 T1	Ta gr3	3 Ta	Sampling visit		Ta	Ta	Ta
	112-10	7 Ta, 2 T1	Ta gr3	2 Ta, 4 T1	Previous visit		Ta	Ta	Ta
	320-7	1 Ta, 2 T1	Ta gr3	2 Ta	Sampling visit		T2	T2	Ta
	967-3	2 T1	Ta gr3	1 T1	Sampling visit		Ta	Ta	Ta
	T1 grade III tumours – no prior muscle invasive tumour								
	625-1		T1 gr3		No		T1	T1	T1
	847-1		T1 gr3		No		T1	T1	T1
	1257-1		T1 gr3		Sampling visit		T1	T1	T1
	919-1		T1 gr3		No		T1	T1	T1
	880-1		T1 gr3	4 Ta	No		T1	T1	T1
	812-1		T1 gr3		No		T1	T1	T1
	1269-1		T1 gr3		No	No review	T1	T1	T1
	1083-2	1 Ta	T1 gr3		No	No review	T1	T1	T1
	1238-1		T1 gr3	1 Ta, 1 T2+	No		T1	T1	T1
	1085-1		T1 gr3		Subsequent visit	No review	T1	T1	T1
	1134-1		T1 gr3	3 T1	Sampling visit	T2 gr3	T1	T1	T1
	T2+ grade III/IV tumours – only primary tumours								
	1164-1		T2+ gr4		No	T2+ gr3	T2/T1	T1	T1
	1032-1		T2+ gr?		ND	No review	T2	T2	T2
	1117-1		T2+ gr3		ND		T2	T2	*T1*
	1178-1		T2+ gr3		ND		T2	T2	T2

1078-1	T2+ gr3		ND		T2	T2	T2
875-1	T2+ gr3		No		T2	T2	T2
1044-1	T2+ gr3	1 T2+	ND		T2	T2	T2
1133-1	T2+ gr3		ND		T2	T2	T2
1068-1	T2+ gr3		No		T2	T2	T2
937-1	T2+ gr3		ND	No review	T1	T1	T1

[a] Examples of tumour histology.

[b] Carcinoma *in situ* detected in selected site biopsies at the time of sampling tumour tissue for the arrays or at previous or subsequent visits.

[c] All tumours were reviewed by a single uro-pathologist and any change compared to the routine classification is listed.

[d] Molecular classification based on 320, 80, and 20 genes cross-validation loops.

Classification using other gene-sets

Classification was also carried out using other gene-sets (10, 20, 32, 40, 80, 160, and 320 genes). These gene-sets demonstrated the same classification tendency as the 71 genes. See Tables 9 - 15 for gene-sets.

Table 9. 320 genes for classifier

Chip acc. #	UniGene Build 162	description
AB000220_at	Hs.171921	NM_006379; semaphorin 3C

Chip acc. #	UniGene Build 162	description
AB000220_at	Hs.171921	NM_006379; semaphorin 3C
AC002073_cds1_at		
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D10922_s_at	Hs.99855	NM_001462; formyl peptide receptor-like 1
D10925_at	Hs.301921	NM_001295; chemokine (C-C motif) receptor 1
D11086_at	Hs.84	NM_000206; interleukin 2 receptor, gamma chain, precursor
D11151_at	Hs.211202	NM_001957; endothelin receptor type A
D13435_at	Hs.426142	NM_002643; phosphatidylinositol glycan, class F isoform 1 NM_173074; phos-

		phatidylinositol glycan, class F isoform 2
D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasciclin I-like)
D14520_at	Hs.84728	NM_001730; Kruppel-like factor 5
D21878_at	Hs.169998	NM_004334; bone marrow stromal cell antigen 1 precursor
D26443_at	Hs.371369	NM_004172; solute carrier family 1 (glial high affinity glutamate transporter), member 3
D28589_at	Hs.17719	
D42046_at	Hs.194665	
D45370_at	Hs.74120	NM_006829; adipose specific 2
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D50495_at	Hs.224397	NM_003195; transcription elongation factor A (SII), 2
D63135_at	Hs.27935	NM_032646; tweety homolog 2
D64053_at	Hs.198288	NM_002849; protein tyrosine phosphatase, receptor type, R isoform 1 precursor NM_130846; protein tyrosine phosphatase, receptor type, R isoform 2
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D85131_s_at	Hs.433881	NM_002383; MYC-associated zinc finger protein
D86062_s_at	Hs.413482	NM_004649; chromosome 21 open reading frame 33
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D86957_at	Hs.307944	
D86959_at	Hs.105751	NM_014720; Ste20-related serine/threonine

		kinase
D86976_at	Hs.196914	
D87433_at	Hs.301989	NM_015136; stabilin 1
D87443_at	Hs.409862	NM_014758; sorting nexin 19
D87682_at	Hs.134792	
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
D90279_s_at	Hs.433695	NM_000093; alpha 1 type V collagen prepro-protein
HG1996-HT2044_at		
HG2090-HT2152_s_at		
HG2463-HT2559_at		
HG2994-HT4850_s_at		
HG3044-HT3742_s_at		
HG3187-HT3366_s_at		
HG3342-HT3519_s_at		
HG371-HT26388_s_at		
HG4069-HT4339_s_at		
HG67-HT67_f_at		
HG907-HT907_at		
J02871_s_at	Hs.436317	NM_000779; cyto-chrome P450, family 4, subfamily B, polypep-tide 1
J03040_at	Hs.111779	NM_003118; secreted protein, acidic, cysteine-rich (osteonectin)
J03060_at		
J03068_at		
J03241_s_at	Hs.2025	NM_003239; transform-ing growth factor, beta 3
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor
J03909_at		
J03925_at	Hs.172631	NM_000632; integrin alpha M precursor
J04056_at	Hs.88778	NM_001757; carbonyl reductase 1
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide
J04093_s_at	Hs.278896	NM_019075; UDP

		glycosyltransferase 1 family, polypeptide A10
J04130_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
J04152_rna1_s_at		
J04162_at	Hs.372679	NM_000569; Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
J04456_at	Hs.407909	NM_002305; beta-galactosidase binding lectin precursor
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05036_s_at	Hs.1355	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
J05448_at	Hs.79402	NM_002694; DNA directed RNA polymerase II polypeptide C NM_032940; DNA directed RNA polymerase II polypeptide C
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
K03430_at		
L06797_s_at	Hs.421986	NM_003467; chemokine (C-X-C motif) receptor 4
L10343_at	Hs.112341	NM_002638; skin-derived protease inhibitor 3 preproprotein
L11708_at	Hs.155109	NM_002153; hydroxysteroid (17-beta) dehydrogenase 2
L13391_at	Hs.78944	NM_002923; regulator of G-protein signalling 2, 24kDa
L13698_at	Hs.65029	NM_002048; growth arrest-specific 1

L13720_at	Hs.437710	NM_000820; growth arrest-specific 6
L13923_at	Hs.750	NM_000138; fibrillin 1
AB000220_at	Hs.171921	NM_006379; sema-phorin 3C
AC002073_cds1_at		
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D10922_s_at	Hs.99855	NM_001462; formyl peptide receptor-like 1
D10925_at	Hs.301921	NM_001295; chemokine (C-C motif) receptor 1
D11086_at	Hs.84	NM_000206; interleukin 2 receptor, gamma chain, precursor
D11151_at	Hs.211202	NM_001957; endothelin receptor type A
D13435_at	Hs.426142	NM_002643; phos-phatidylinositol glycan, class F isoform 1 NM_173074; phos-phatidylinositol glycan, class F isoform 2
D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasci-clin I-like)
D14520_at	Hs.84728	NM_001730; Kruppel-like factor 5
D21878_at	Hs.169998	NM_004334; bone marrow stromal cell antigen 1 precursor
D26443_at	Hs.371369	NM_004172; solute carrier family 1 (glial high affinity glutamate transporter), member 3
D28589_at	Hs.17719	
D42046_at	Hs.194665	
D45370_at	Hs.74120	NM_006829; adipose specific 2
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D50495_at	Hs.224397	NM_003195; transcrip-tion elongation factor A (SII), 2
D63135_at	Hs.27935	NM_032646; tweety

		homolog 2
D64053_at	Hs.198288	NM_002849; protein tyrosine phosphatase, receptor type, R isoform 1 precursor NM_130846; protein tyrosine phosphatase, receptor type, R isoform 2
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D85131_s_at	Hs.433881	NM_002383; MYC-associated zinc finger protein
D86062_s_at	Hs.413482	NM_004649; chromosome 21 open reading frame 33
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D86957_at	Hs.307944	
D86959_at	Hs.105751	NM_014720; Ste20-related serine/threonine kinase
D86976_at	Hs.196914	
D87433_at	Hs.301989	NM_015136; stabilin 1
D87443_at	Hs.409862	NM_014758; sorting nexin 19
D87682_at	Hs.134792	
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
D90279_s_at	Hs.433695	NM_000093; alpha 1 type V collagen preproprotein
HG1996-HT2044_at		
HG2090-HT2152_s_at		
HG2463-HT2559_at		
HG2994-HT4850_s_at		
HG3044-HT3742_s_at		
HG3187-HT3366_s_at		
HG3342-HT3519_s_at		
HG371-HT26388_s_at		
HG4069-HT4339_s_at		
HG67-HT67_f_at		
HG907-HT907_at		

J02871_s_at	Hs.436317	NM_000779; cytochrome P450, family 4, subfamily B, polypeptide 1
J03040_at	Hs.111779	NM_003118; secreted protein, acidic, cysteine-rich (osteonectin)
J03060_at		
J03068_at		
J03241_s_at	Hs.2025	NM_003239; transforming growth factor, beta 3
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor
J03909_at		
J03925_at	Hs.172631	NM_000632; integrin alpha M precursor
J04056_at	Hs.88778	NM_001757; carbonyl reductase 1
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide
J04093_s_at	Hs.278896	NM_019075; UDP glycosyltransferase 1 family, polypeptide A10
J04130_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
J04152_rna1_s_at		
J04162_at	Hs.372679	NM_000569; Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
J04456_at	Hs.407909	NM_002305; beta-galactosidase binding lectin precursor
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05036_s_at	Hs.1355	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
J05448_at	Hs.79402	NM_002694; DNA directed RNA poly-

		merase II polypeptide C NM_032940; DNA directed RNA polymerase II polypeptide C
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
K03430_at		
L06797_s_at	Hs.421986	NM_003467; chemokine (C-X-C motif) receptor 4
L10343_at	Hs.112341	NM_002638; skin-derived protease inhibitor 3 preproprotein
L11708_at	Hs.155109	NM_002153; hydroxysteroid (17-beta) dehydrogenase 2
L13391_at	Hs.78944	NM_002923; regulator of G-protein signalling 2, 24kDa
L13698_at	Hs.65029	NM_002048; growth arrest-specific 1
L13720_at	Hs.437710	NM_000820; growth arrest-specific 6
L13923_at	Hs.750	NM_000138; fibrillin 1
AB000220_at	Hs.171921	NM_006379; semaphorin 3C
AC002073_cds1_at		
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D10922_s_at	Hs.99855	NM_001462; formyl peptide receptor-like 1
D10925_at	Hs.301921	NM_001295; chemokine (C-C motif) receptor 1
D11086_at	Hs.84	NM_000206; interleukin 2 receptor, gamma chain, precursor
D11151_at	Hs.211202	NM_001957; endothelin receptor type A
D13435_at	Hs.426142	NM_002643; phosphatidylinositol glycan, class F isoform 1 NM_173074; phosphatidylinositol glycan, class F isoform 2

D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasciclin I-like)
D14520_at	Hs.84728	NM_001730; Kruppel-like factor 5
D21878_at	Hs.169998	NM_004334; bone marrow stromal cell antigen 1 precursor
D26443_at	Hs.371369	NM_004172; solute carrier family 1 (glial high affinity glutamate transporter), member 3
D28589_at	Hs.17719	
D42046_at	Hs.194665	
D45370_at	Hs.74120	NM_006829; adipose specific 2
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D50495_at	Hs.224397	NM_003195; transcription elongation factor A (SII), 2
D63135_at	Hs.27935	NM_032646; tweety homolog 2
D64053_at	Hs.198288	NM_002849; protein tyrosine phosphatase, receptor type, R isoform 1 precursor NM_130846; protein tyrosine phosphatase, receptor type, R isoform 2
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D85131_s_at	Hs.433881	NM_002383; MYC-associated zinc finger protein
D86062_s_at	Hs.413482	NM_004649; chromosome 21 open reading frame 33
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D86957_at	Hs.307944	
D86959_at	Hs.105751	NM_014720; Ste20-related serine/threonine kinase
D86976_at	Hs.196914	

D87433_at	Hs.301989	NM_015136; stabilin 1
D87443_at	Hs.409862	NM_014758; sorting nexin 19
D87682_at	Hs.134792	
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
D90279_s_at	Hs.433695	NM_000093; alpha 1 type V collagen prepro-protein
HG1996-HT2044_at		
HG2090-HT2152_s_at		
HG2463-HT2559_at		
HG2994-HT4850_s_at		
HG3044-HT3742_s_at		
HG3187-HT3366_s_at		
HG3342-HT3519_s_at		
HG371-HT26388_s_at		
HG4069-HT4339_s_at		
HG67-HT67_f_at		
HG907-HT907_at		
J02871_s_at	Hs.436317	NM_000779; cyto-chrome P450, family 4, subfamily B, polypep-tide 1
J03040_at	Hs.111779	NM_003118; secreted protein, acidic, cysteine-rich (osteonectin)
J03060_at		
J03068_at		
J03241_s_at	Hs.2025	NM_003239; transform-ing growth factor, beta 3
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor
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J03925_at	Hs.172631	NM_000632; integrin alpha M precursor
J04056_at	Hs.88778	NM_001757; carbonyl reductase 1
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide
J04093_s_at	Hs.278896	NM_019075; UDP glycosyltransferase 1 family, polypeptide A10

J04130_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
J04152_ma1_s_at		
J04162_at	Hs.372679	NM_000569; Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
J04456_at	Hs.407909	NM_002305; beta-galactosidase binding lectin precursor
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05036_s_at	Hs.1355	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
J05448_at	Hs.79402	NM_002694; DNA directed RNA polymerase II polypeptide C NM_032940; DNA directed RNA polymerase II polypeptide C
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
K03430_at		
L06797_s_at	Hs.421986	NM_003467; chemokine (C-X-C motif) receptor 4
L10343_at	Hs.112341	NM_002638; skin-derived protease inhibitor 3 preproprotein
L11708_at	Hs.155109	NM_002153; hydroxysteroid (17-beta) dehydrogenase 2
L13391_at	Hs.78944	NM_002923; regulator of G-protein signalling 2, 24kDa
L13698_at	Hs.65029	NM_002048; growth arrest-specific 1
L13720_at	Hs.437710	NM_000820; growth arrest-specific 6

L13923_at	Hs.750	NM_000138; fibrillin 1
AB000220_at	Hs.171921	NM_006379; sema-phorin 3C
AC002073_cds1_at		
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D10922_s_at	Hs.99855	NM_001462; formyl peptide receptor-like 1
D10925_at	Hs.301921	NM_001295; chemokine (C-C motif) receptor 1
D11086_at	Hs.84	NM_000206; interleukin 2 receptor, gamma chain, precursor
D11151_at	Hs.211202	NM_001957; endothelin receptor type A
D13435_at	Hs.426142	NM_002643; phos-phatidylinositol glycan, class F isoform 1 NM_173074; phos-phatidylinositol glycan, class F isoform 2
D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasci-clin I-like)
D14520_at	Hs.84728	NM_001730; Kruppel-like factor 5
D21878_at	Hs.169998	NM_004334; bone marrow stromal cell antigen 1 precursor
D26443_at	Hs.371369	NM_004172; solute carrier family 1 (glial high affinity glutamate transporter), member 3
D28589_at	Hs.17719	
D42046_at	Hs.194665	
D45370_at	Hs.74120	NM_006829; adipose specific 2
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D50495_at	Hs.224397	NM_003195; transcrip-tion elongation factor A (SII), 2
D63135_at	Hs.27935	NM_032646; tweety homolog 2
D64053_at	Hs.198288	NM_002849; protein

		tyrosine phosphatase, receptor type, R isoform 1 precursor NM_130846; protein tyrosine phosphatase, receptor type, R isoform 2
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D85131_s_at	Hs.433881	NM_002383; MYC-associated zinc finger protein
D86062_s_at	Hs.413482	NM_004649; chromosome 21 open reading frame 33
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D86957_at	Hs.307944	
D86959_at	Hs.105751	NM_014720; Ste20-related serine/threonine kinase
D86976_at	Hs.196914	
D87433_at	Hs.301989	NM_015136; stabilin 1
D87443_at	Hs.409862	NM_014758; sorting nexin 19
D87682_at	Hs.134792	
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
D90279_s_at	Hs.433695	NM_000093; alpha 1 type V collagen preproprotein
HG1996-HT2044_at		
HG2090-HT2152_s_at		
HG2463-HT2559_at		
HG2994-HT4850_s_at		
HG3044-HT3742_s_at		
HG3187-HT3366_s_at		
HG3342-HT3519_s_at		
HG371-HT26388_s_at		
HG4069-HT4339_s_at		
HG67-HT67_f_at		
HG907-HT907_at		
J02871_s_at	Hs.436317	NM_000779; cytochrome P450, family 4,

		subfamily B, polypeptide 1
J03040_at	Hs.111779	NM_003118; secreted protein, acidic, cysteine-rich (osteonectin)
J03060_at		
J03068_at		
J03241_s_at	Hs.2025	NM_003239; transforming growth factor, beta 3
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor
J03909_at		
J03925_at	Hs.172631	NM_000632; integrin alpha M precursor
J04056_at	Hs.88778	NM_001757; carbonyl reductase 1
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide
J04093_s_at	Hs.278896	NM_019075; UDP glycosyltransferase 1 family, polypeptide A10
J04130_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
J04152_rna1_s_at		
J04162_at	Hs.372679	NM_000569; Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
J04456_at	Hs.407909	NM_002305; beta-galactosidase binding lectin precursor
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05036_s_at	Hs.1355	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
J05448_at	Hs.79402	NM_002694; DNA directed RNA polymerase II polypeptide C NM_032940; DNA

		directed RNA polymerase II polypeptide C
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
K03430_at		
L06797_s_at	Hs.421986	NM_003467; chemokine (C-X-C motif) receptor 4
L10343_at	Hs.112341	NM_002638; skin-derived protease inhibitor 3 preproprotein
L11708_at	Hs.155109	NM_002153; hydroxysteroid (17-beta) dehydrogenase 2
L13391_at	Hs.78944	NM_002923; regulator of G-protein signalling 2, 24kDa
L13698_at	Hs.65029	NM_002048; growth arrest-specific 1
L13720_at	Hs.437710	NM_000820; growth arrest-specific 6
L13923_at	Hs.750	NM_000138; fibrillin 1
AB000220_at	Hs.171921	NM_006379; semaphorin 3C
AC002073_cds1_at		
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D10922_s_at	Hs.99855	NM_001462; formyl peptide receptor-like 1
D10925_at	Hs.301921	NM_001295; chemokine (C-C motif) receptor 1
D11086_at	Hs.84	NM_000206; interleukin 2 receptor, gamma chain, precursor
D11151_at	Hs.211202	NM_001957; endothelin receptor type A
D13435_at	Hs.426142	NM_002643; phosphatidylinositol glycan, class F isoform 1 NM_173074; phosphatidylinositol glycan, class F isoform 2
D13666_s_at	Hs.136348	NM_006475; osteoblast

		specific factor 2 (fasciclin I-like)
D14520_at	Hs.84728	NM_001730; Kruppel-like factor 5
D21878_at	Hs.169998	NM_004334; bone marrow stromal cell antigen 1 precursor
D26443_at	Hs.371369	NM_004172; solute carrier family 1 (glial high affinity glutamate transporter), member 3
D28589_at	Hs.17719	
D42046_at	Hs.194665	
D45370_at	Hs.74120	NM_006829; adipose specific 2
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D50495_at	Hs.224397	NM_003195; transcription elongation factor A (SII), 2
D63135_at	Hs.27935	NM_032646; tweety homolog 2
D64053_at	Hs.198288	NM_002849; protein tyrosine phosphatase, receptor type, R isoform 1 precursor NM_130846; protein tyrosine phosphatase, receptor type, R isoform 2
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D85131_s_at	Hs.433881	NM_002383; MYC-associated zinc finger protein
D86062_s_at	Hs.413482	NM_004649; chromosome 21 open reading frame 33
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D86957_at	Hs.307944	
D86959_at	Hs.105751	NM_014720; Ste20-related serine/threonine kinase
D86976_at	Hs.196914	

D87433_at	Hs.301989	NM_015136; stabilin 1
D87443_at	Hs.409862	NM_014758; sorting nexin 19
D87682_at	Hs.134792	
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
D90279_s_at	Hs.433695	NM_000093; alpha 1 type V collagen prepro-protein
HG1996-HT2044_at		
HG2090-HT2152_s_at		
HG2463-HT2559_at		
HG2994-HT4850_s_at		

Table 10. 160 Genes for classifier

Chip acc. #	UniGene Build 162	description
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasciclin I-like)
D21878_at	Hs.169998	NM_004334; bone marrow stromal cell antigen 1 precursor
D45370_at	Hs.74120	NM_006829; adipose specific 2
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D85131_s_at	Hs.433881	NM_002383; MYC-associated zinc finger protein
D86062_s_at	Hs.413482	NM_004649; chromosome 21 open reading frame 33
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D86957_at	Hs.307944	
D86976_at	Hs.196914	
D87433_at	Hs.301989	NM_015136; stabilin 1
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
HG3044-HT3742_s_at		
HG371-HT26388_s_at		
HG4069-HT4339_s_at		
HG67-HT67_f_at		
HG907-HT907_at		
J02871_s_at	Hs.436317	NM_000779; cytochrome P450, family 4, subfamily B, polypeptide 1
J03040_at	Hs.111779	NM_003118; secreted protein, acidic, cysteine-rich (osteonectin)
J03068_at		
J03241_s_at	Hs.2025	NM_003239; transforming growth factor, beta 3
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor
J03909_at		
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide

J04130_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
J04162_at	Hs.372679	NM_000569; Fc fragment of IgG, low affinity IIIa, receptor for (CD16)
J04456_at	Hs.407909	NM_002305; beta-galactosidase binding lectin precursor
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
J05448_at	Hs.79402	NM_002694; DNA directed RNA polymerase II polypeptide C NM_032940; DNA directed RNA polymerase II polypeptide C
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
K03430_at		
L13698_at	Hs.65029	NM_002048; growth arrest-specific 1
L13720_at	Hs.437710	NM_000820; growth arrest-specific 6
L13923_at	Hs.750	NM_000138; fibrillin 1
L15409_at	Hs.421597	NM_000551; elogin binding protein
L17325_at	Hs.195825	NM_006867; RNA-binding protein with multiple splicing
L19872_at	Hs.170087	NM_001621; aryl hydrocarbon receptor
L27476_at	Hs.75608	NM_004817; tight junction protein 2 (zona occludens 2)
L33799_at	Hs.202097	NM_002593; procollagen C-endopeptidase enhancer
L40388_at	Hs.30212	NM_004236; thyroid receptor interacting protein 15
L40904_at	Hs.387667	NM_005037; peroxisome proliferative activated receptor gamma isoform 1 NM_015869; peroxisome proliferative activated receptor gamma isoform 2 NM_138711; peroxisome proliferative activated receptor gamma isoform 1 NM_138712; peroxisome proliferative activated receptor gamma isoform 1
L41919_ma1_at		
M11433_at	Hs.101850	NM_002899; retinol binding protein 1, cellular
M11718_at	Hs.283393	NM_000393; alpha 2 type V collagen preproprotein
M12125_at	Hs.300772	NM_003289; tropomyosin 2 (beta)
M14218_at	Hs.442047	NM_000048; argininosuccinate lyase
M15395_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
M16591_s_at	Hs.89555	NM_002110; hemopoietic cell kinase isoform p61HCK
M17219_at	Hs.203862	NM_002069; guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1
M20530_at		
M23178_s_at	Hs.73817	NM_002983; chemokine (C-C motif) ligand 3
M28130_ma1_s_at		
M29550_at	Hs.187543	NM_021132; protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)
M31165_at	Hs.407546	NM_007115; tumor necrosis factor, alpha-induced protein 6 precursor
M32011_at	Hs.949	NM_000433; neutrophil cytosolic factor 2
M33195_at	Hs.433300	NM_004106; Fc fragment of IgE, high affinity I, receptor for, gamma polypeptide precursor
M37033_at	Hs.443057	NM_000560; CD53 antigen
M37766_at	Hs.901	NM_001778; CD48 antigen (B-cell membrane protein)
M55998_s_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

M57731_s_at	Hs.75765	NM_002089; chemokine (C-X-C motif) ligand 2
M62840_at	Hs.82542	NM_001637; acyloxyacyl hydrolase precursor
M63262_at		
M68840_at	Hs.183109	NM_000240; monoamine oxidase A
M69203_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
M72885_rna1_s_at		
M77349_at	Hs.421496	NM_000358; transforming growth factor, beta-induced, 68kDa
M82882_at	Hs.124030	NM_172373; E74-like factor 1 (ets domain transcription factor)
M83822_at	Hs.209846	NM_006726; LPS-responsive vesicle trafficking, beach and anchor containing
M92934_at	Hs.410037	NM_001901; connective tissue growth factor
M95178_at	Hs.119000	NM_001102; actinin, alpha 1
S69115_at	Hs.10306	NM_005601; natural killer cell group 7 sequence
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)
S78187_at	Hs.153752	NM_004358; cell division cycle 25B isoform 1 NM_021872; cell division cycle 25B isoform 2 NM_021873; cell division cycle 25B isoform 3 NM_021874; cell division cycle 25B isoform 4
U01833_at	Hs.81469	NM_002484; nucleotide binding protein 1 (MinD homolog, E. coli)
U07231_at	Hs.309763	NM_002092; G-rich RNA sequence binding factor 1
U09278_at	Hs.436852	NM_004460; fibroblast activation protein, alpha subunit
U09937_rna1_s_at		
U10550_at	Hs.79022	NM_005261; GTP-binding mitogen-induced T-cell protein NM_181702; GTP-binding mitogen-induced T-cell protein
U12424_s_at	Hs.108646	NM_000408; glycerol-3-phosphate dehydrogenase 2 (mitochondrial)
U16306_at	Hs.434488	NM_004385; chondroitin sulfate proteoglycan 2 (versican)
U20158_at	Hs.2488	NM_005565; lymphocyte cytosolic protein 2
U20536_s_at	Hs.3280	NM_001226; caspase 6 isoform alpha preproprotein NM_032992; caspase 6 isoform beta
U24266_at	Hs.77448	NM_003748; aldehyde dehydrogenase 4A1 precursor NM_170726; aldehyde dehydrogenase 4A1 precursor
U28249_at	Hs.301350	NM_005971; FXYD domain containing ion transport regulator 3 isoform 1 precursor NM_021910; FXYD domain containing ion transport regulator 3 isoform 2 precursor
U28488_s_at	Hs.155935	NM_004054; complement component 3a receptor 1
U29680_at	Hs.227817	NM_004049; BCL2-related protein A1
U37143_at	Hs.152096	NM_000775; cytochrome P450, family 2, subfamily J, polypeptide 2
U38864_at	Hs.108139	NM_012256; zinc finger protein 212
U39840_at	Hs.163484	NM_004496; forkhead box A1
U41315_rna1_s_at		
U44111_at	Hs.42151	NM_006895; histamine N-methyltransferase
U47414_at	Hs.13291	NM_004354; cyclin G2
U49352_at	Hs.414754	NM_001359; 2,4-dienoyl CoA reductase 1 precursor
U50708_at	Hs.1265	NM_000056; branched chain keto acid dehydrogenase E1, beta polypeptide precursor NM_183050; branched chain keto acid dehydrogenase E1, beta polypeptide precursor

U52101_at	Hs.9999	NM_001425; epithelial membrane protein 3
U59914_at	Hs.153863	NM_005585; MAD, mothers against decapentaplegic homolog 6
U60205_at	Hs.393239	NM_006745; sterol-C4-methyl oxidase-like
U61981_at	Hs.42674	NM_002439; mutS homolog 3
U64520_at	Hs.66708	NM_004781; vesicle-associated membrane protein 3 (cellubrevin)
U65093_at	Hs.82071	NM_006079; Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
U66619_at	Hs.444445	NM_003078; SWI/SNF-related matrix-associated actin-dependent regulator of chromatin d3
U68019_at	Hs.288261	NM_005902; MAD, mothers against decapentaplegic homolog 3
U68385_at	Hs.380923	
U68485_at	Hs.193163	NM_004305; bridging integrator 1 isoform 8 NM_139343; bridging integrator 1 isoform 1 NM_139344; bridging integrator 1 isoform 2 NM_139345; bridging integrator 1 isoform 3 NM_139346; bridging integrator 1 isoform 4 NM_139347; bridging integrator 1 isoform 5 NM_139348; bridging integrator 1 isoform 6 NM_139349; bridging integrator 1 isoform 7 NM_139350; bridging integrator 1 isoform 9 NM_139351; bridging integrator 1 isoform 10
U74324_at	Hs.90875	NM_002871; RAB-interacting factor
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;
U83303_cds2_at	Hs.164021	NM_002993; chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
U88871_at	Hs.79993	NM_000288; peroxisomal biogenesis factor 7
U90549_at	Hs.236774	NM_006353; high mobility group nucleosomal binding domain 4
U90716_at	Hs.79187	NM_001338; coxsackie virus and adenovirus receptor
V00594_at	Hs.118786	NM_005953; metallothionein 2A
V00594_s_at	Hs.118786	NM_005953; metallothionein 2A
X02761_s_at	Hs.418138	NM_002026; fibronectin 1 isoform 1 preproprotein NM_054034; fibronectin 1 isoform 2 preproprotein
X04011_at	Hs.88974	NM_000397; cytochrome b-245, beta polypeptide (chronic granulomatous disease)
X04085_rna1_at		
X07438_s_at		
X07743_at	Hs.77436	NM_002664; pleckstrin
X13334_at	Hs.75627	NM_000591; CD14 antigen precursor
X14046_at	Hs.153053	NM_001774; CD37 antigen
X14813_at	Hs.166160	NM_001607; acetyl-Coenzyme A acyltransferase 1
X15880_at	Hs.415997	NM_001848; collagen, type VI, alpha 1 precursor
X15882_at	Hs.420269	NM_001849; alpha 2 type VI collagen isoform 2C2 precursor NM_058174; alpha 2 type VI collagen isoform 2C2a precursor NM_058175; alpha 2 type VI collagen isoform 2C2a precursor
X51408_at	Hs.380138	NM_001822; chimerin (chimaerin) 1
X53800_s_at	Hs.89690	NM_002090; chemokine (C-X-C motif) ligand 3
X54489_rna1_at		
X57351_s_at	Hs.174195	NM_006435; interferon induced transmembrane protein 2 (1-8D)
X57579_s_at		
X58072_at	Hs.169946	NM_002051; GATA binding protein 3 NM_032742;

X62048_at	Hs.249441	NM_003390; wee1 tyrosine kinase
X64072_s_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
X65614_at	Hs.2962	NM_005980; S100 calcium binding protein P
X66945_at	Hs.748	NM_000604; fibroblast growth factor receptor 1 isoform 1 precursor NM_015850; fibroblast growth factor receptor 1 isoform 2 precursor NM_023105; fibroblast growth factor receptor 1 isoform 3 precursor NM_023106; fibroblast growth factor receptor 1 isoform 4 precursor NM_023107; fibroblast growth factor receptor 1 isoform 5 precursor NM_023108; fibroblast growth factor receptor 1 isoform 6 precursor NM_023109; fibroblast growth factor receptor 1 isoform 7 precursor NM_023110; fibroblast growth factor receptor 1 isoform 8 precursor NM_023111; fibroblast growth factor receptor 1 isoform 9 precursor
X67491_f_at	Hs.355697	NM_005271; glutamate dehydrogenase 1
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7
X78520_at	Hs.372528	NM_001829; chloride channel 3
X78549_at	Hs.51133	NM_005975; PTK6 protein tyrosine kinase 6
X78565_at	Hs.98998	NM_002160; tenascin C (hexabrachion)
X78669_at	Hs.79088	NM_002902; reticulocalbin 2, EF-hand calcium binding domain
X83618_at	Hs.59889	NM_005518; 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)
X84908_at	Hs.78060	NM_000293; phosphorylase kinase, beta
X90908_at	Hs.147391	NM_001445; gastrotropin
X91504_at	Hs.389277	NM_003224; ADP-ribosylation factor related protein 1
X95632_s_at	Hs.387906	NM_005759; abl-interactor 2
X97267_rna1_s_at		
Y00705_at	Hs.407856	NM_003122; serine protease inhibitor, Kazal type 1
Y00787_s_at	Hs.624	NM_000584; interleukin 8 precursor
Y00815_at	Hs.75216	NM_002840; protein tyrosine phosphatase, receptor type, F isoform 1 precursor NM_130440; protein tyrosine phosphatase, receptor type, F isoform 2 precursor
Y08374_rna1_at		
Z12173_at	Hs.334534	NM_002076; glucosamine (N-acetyl)-6-sulfatase precursor
Z19554_s_at	Hs.435800	NM_003380; vimentin
Z26491_s_at	Hs.240013	NM_000754; catechol-O-methyltransferase isoform MB-COMT NM_007310; catechol-O-methyltransferase isoform S-COMT
Z29331_at	Hs.372758	NM_003344; ubiquitin-conjugating enzyme E2H isoform 1 NM_182697; ubiquitin-conjugating enzyme E2H isoform 2
Z35491_at	Hs.377484	NM_004323; BCL2-associated athanogene isoform 1L
Z48199_at	Hs.82109	NM_002997; syndecan 1
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865; NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

Table 11. 80 genes for classifier

Chip acc. #	UniGene Build 162	description
AF000231_at	Hs.75618	NM_004663; Ras-related protein Rab-11A
D13666_s_at	Hs.136348	NM_006475; osteoblast specific factor 2 (fasciclin I-like)
D49372_s_at	Hs.54460	NM_002986; small inducible cytokine A11 precursor
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D86479_at	Hs.439463	NM_001129; adipocyte enhancer binding protein 1 precursor
D87433_at	Hs.301989	NM_015136; stabilin 1
D89077_at	Hs.75367	NM_006748; Src-like-adaptor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
HG4069-HT4339_s_at		
HG67-HT67_f_at		
HG907-HT907_at		
J02871_s_at	Hs.436317	NM_000779; cytochrome P450, family 4, subfamily B, polypeptide 1
J03278_at	Hs.307783	NM_002609; platelet-derived growth factor receptor beta precursor
J04058_at	Hs.169919	NM_000126; electron transfer flavoprotein, alpha polypeptide
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
J05448_at	Hs.79402	NM_002694; DNA directed RNA polymerase II polypeptide C NM_032940; DNA directed RNA polymerase II polypeptide C
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
L13720_at	Hs.437710	NM_000820; growth arrest-specific 6
L40904_at	Hs.387667	NM_005037; peroxisome proliferative activated receptor gamma isoform 1 NM_015869; peroxisome proliferative activated receptor gamma isoform 2 NM_138711; peroxisome proliferative activated receptor gamma isoform 1 NM_138712; peroxisome proliferative activated receptor gamma isoform 1
M12125_at	Hs.300772	NM_003289; tropomyosin 2 (beta)
M15395_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
M16591_s_at	Hs.89555	NM_002110; hemopoietic cell kinase isoform p61HCK
M20530_at		
M23178_s_at	Hs.73817	NM_002983; chemokine (C-C motif) ligand 3
M32011_at	Hs.949	NM_000433; neutrophil cytosolic factor 2
M33195_at	Hs.433300	NM_004106; Fc fragment of IgE, high affinity I, receptor for, gamma polypeptide precursor
M55998_s_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein
M57731_s_at	Hs.75765	NM_002089; chemokine (C-X-C motif) ligand 2
M63262_at		
M68840_at	Hs.183109	NM_000240; monoamine oxidase A
M69203_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
M72885_ma1_s_at		
M83822_at	Hs.209846	NM_006726; LPS-responsive vesicle trafficking, beach and anchor

		containing
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)
U01833_at	Hs.81469	NM_002484; nucleotide binding protein 1 (MinD homolog, E. coli)
U07231_at	Hs.309763	NM_002092; G-rich RNA sequence binding factor 1
U09937_rna1_s_at		
U10550_at	Hs.79022	NM_005261; GTP-binding mitogen-induced T-cell protein NM_181702; GTP-binding mitogen-induced T-cell protein
U20158_at	Hs.2488	NM_005565; lymphocyte cytosolic protein 2
U28488_s_at	Hs.155935	NM_004054; complement component 3a receptor 1
U29680_at	Hs.227817	NM_004049; BCL2-related protein A1
U41315_rna1_s_at		
U47414_at	Hs.13291	NM_004354; cyclin G2
U49352_at	Hs.414754	NM_001359; 2,4-dienoyl CoA reductase 1 precursor
U50708_at	Hs.1265	NM_000056; branched chain keto acid dehydrogenase E1, beta polypeptide precursor NM_183050; branched chain keto acid dehydrogenase E1, beta polypeptide precursor
U52101_at	Hs.9999	NM_001425; epithelial membrane protein 3
U59914_at	Hs.153863	NM_005585; MAD, mothers against decapentaplegic homolog 6
U64520_at	Hs.66708	NM_004781; vesicle-associated membrane protein 3 (cellubrevin)
U65093_at	Hs.82071	NM_006079; Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
U68019_at	Hs.288261	NM_005902; MAD, mothers against decapentaplegic homolog 3
U68385_at	Hs.380923	
U74324_at	Hs.90875	NM_002871; RAB-interacting factor
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;
U90549_at	Hs.236774	NM_006353; high mobility group nucleosomal binding domain 4
X04085_rna1_at		
X07438_s_at		
X07743_at	Hs.77436	NM_002664; pleckstrin
X13334_at	Hs.75627	NM_000591; CD14 antigen precursor
X14046_at	Hs.153053	NM_001774; CD37 antigen
X15880_at	Hs.415997	NM_001848; collagen, type VI, alpha 1 precursor
X15882_at	Hs.420269	NM_001849; alpha 2 type VI collagen isoform 2C2 precursor NM_058174; alpha 2 type VI collagen isoform 2C2a precursor NM_058175; alpha 2 type VI collagen isoform 2C2a precursor
X51408_at	Hs.380138	NM_001822; chimerin (chimaerin) 1
X53800_s_at	Hs.89690	NM_002090; chemokine (C-X-C motif) ligand 3
X54489_rna1_at		
X57579_s_at		
X62048_at	Hs.249441	NM_003390; wee1 tyrosine kinase
X64072_s_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
X67491_f_at	Hs.355697	NM_005271; glutamate dehydrogenase 1
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7
X78520_at	Hs.372528	NM_001829; chloride channel 3
X97267_rna1_s_at		

Y00787_s_at	Hs.624	NM_000584; interleukin 8 precursor
Z12173_at	Hs.334534	NM_002076; glucosamine (N-acetyl)-6-sulfatase precursor
Z19554_s_at	Hs.435800	NM_003380; vimentin
Z26491_s_at	Hs.240013	NM_000754; catechol-O-methyltransferase isoform MB-COMT NM_007310; catechol-O-methyltransferase isoform S-COMT
Z29331_at	Hs.372758	NM_003344; ubiquitin-conjugating enzyme E2H isoform 1 NM_182697; ubiquitin-conjugating enzyme E2H isoform 2
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865; NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

Table 12. 40 genes for classifier

Chip acc. #	UniGene Build 162	description
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
J02871_s_at	Hs.436317	NM_000779; cytochrome P450, family 4, subfamily B, polypeptide 1
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
J05070_at	Hs.151738	NM_004994; matrix metalloproteinase 9 preproprotein
M16591_s_at	Hs.89555	NM_002110; hemopoietic cell kinase isoform p61HCK
M23178_s_at	Hs.73817	NM_002983; chemokine (C-C motif) ligand 3
M32011_at	Hs.949	NM_000433; neutrophil cytosolic factor 2
M33195_at	Hs.433300	NM_004106; Fc fragment of IgE, high affinity I, receptor for, gamma polypeptide precursor
M57731_s_at	Hs.75765	NM_002089; chemokine (C-X-C motif) ligand 2
M68840_at	Hs.183109	NM_000240; monoamine oxidase A
M69203_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)
U01833_at	Hs.81469	NM_002484; nucleotide binding protein 1 (MinD homolog, E. coli)
U07231_at	Hs.309763	NM_002092; G-rich RNA sequence binding factor 1
U09937_rna1_s_at		
U20158_at	Hs.2488	NM_005565; lymphocyte cytosolic protein 2
U41315_rna1_s_at		
U47414_at	Hs.13291	NM_004354; cyclin G2
U49352_at	Hs.414754	NM_001359; 2,4-dienoyl CoA reductase 1 precursor
U50708_at	Hs.1265	NM_000056; branched chain keto acid dehydrogenase E1, beta polypeptide precursor NM_183050; branched chain keto acid dehydrogenase E1, beta polypeptide precursor
U65093_at	Hs.82071	NM_006079; Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
U68385_at	Hs.380923	
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;
U90549_at	Hs.236774	NM_006353; high mobility group nucleosomal binding domain 4
X13334_at	Hs.75627	NM_000591; CD14 antigen precursor

X15880_at	Hs.415997	NM_001848; collagen, type VI, alpha 1 precursor
X15882_at	Hs.420269	NM_001849; alpha 2 type VI collagen isoform 2C2 precursor NM_058174; alpha 2 type VI collagen isoform 2C2a precursor NM_058175; alpha 2 type VI collagen isoform 2C2a precursor
X51408_at	Hs.380138	NM_001822; chimerin (chimaerin) 1
X53800_s_at	Hs.89690	NM_002090; chemokine (C-X-C motif) ligand 3
X54489_rna1_at		
X57579_s_at		
X64072_s_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
X67491_f_at	Hs.355697	NM_005271; glutamate dehydrogenase 1
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7
X78520_at	Hs.372528	NM_001829; chloride channel 3
Z29331_at	Hs.372758	NM_003344; ubiquitin-conjugating enzyme E2H isoform 1 NM_182697; ubiquitin-conjugating enzyme E2H isoform 2
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865; NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

Table 13. 20 genes for classifier

Chip acc. #	UniGene Build 162	description
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
M23178_s_at	Hs.73817	NM_002983; chemokine (C-C motif) ligand 3
M32011_at	Hs.949	NM_000433; neutrophil cytosolic factor 2
M69203_s_at	Hs.75703	NM_002984; chemokine (C-C motif) ligand 4 precursor
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)
U07231_at	Hs.309763	NM_002092; G-rich RNA sequence binding factor 1
U41315_rna1_s_at		
U47414_at	Hs.13291	NM_004354; cyclin G2
U49352_at	Hs.414754	NM_001359; 2,4-dienoyl CoA reductase 1 precursor
U50708_at	Hs.1265	NM_000056; branched chain keto acid dehydrogenase E1, beta polypeptide precursor NM_183050; branched chain keto acid dehydrogenase E1, beta polypeptide precursor
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;
X13334_at	Hs.75627	NM_000591; CD14 antigen precursor
X57579_s_at		
X64072_s_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7
X78520_at	Hs.372528	NM_001829; chloride channel 3
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865;

		NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

Table 14. 10 genes for classifier

Chip acc. #	UniGene Build 162	description
D89377_at	Hs.89404	NM_002449; msh homeo box homolog 2
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)
U41315_rna1_s_at		
U47414_at	Hs.13291	NM_004354; cyclin G2
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7
X78520_at	Hs.372528	NM_001829; chloride channel 3
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865; NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

Table 15. 32 genes for classifier

Chip acc. #	UniGene Build 162	description
D83920_at	Hs.440898	NM_002003; ficolin 1 precursor
HG67-HT67_f_at		
HG907-HT907_at		
J05032_at	Hs.32393	NM_001349; aspartyl-tRNA synthetase
K01396_at	Hs.297681	NM_000295; serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
M16591_s_at	Hs.89555	NM_002110; hemopoietic cell kinase isoform p61HCK
M32011_at	Hs.949	NM_000433; neutrophil cytosolic factor 2
M33195_at	Hs.433300	NM_004106; Fc fragment of IgE, high affinity I, receptor for, gamma polypeptide precursor
M37033_at	Hs.443057	NM_000560; CD53 antigen
M57731_s_at	Hs.75765	NM_002089; chemokine (C-X-C motif) ligand 2
M63262_at		
S77393_at	Hs.145754	NM_016531; Kruppel-like factor 3 (basic)
U01833_at	Hs.81469	NM_002484; nucleotide binding protein 1 (MinD homolog, E. coli)
U07231_at	Hs.309763	NM_002092; G-rich RNA sequence binding factor 1
U41315_rna1_s_at		
U47414_at	Hs.13291	NM_004354; cyclin G2
U50708_at	Hs.1265	NM_000056; branched chain keto acid dehydrogenase E1, beta polypeptide precursor NM_183050; branched chain keto acid dehydrogenase E1, beta polypeptide precursor
U52101_at	Hs.9999	NM_001425; epithelial membrane protein 3

U74324_at	Hs.90875	NM_002871; RAB-interacting factor
U77970_at	Hs.321164	NM_002518; neuronal PAS domain protein 2 NM_032235;
U90549_at	Hs.236774	NM_006353; high mobility group nucleosomal binding domain 4
X13334_at	Hs.75627	NM_000591; CD14 antigen precursor
X54489_rna1_at		
X57579_s_at		
X64072_s_at	Hs.375957	NM_000211; integrin beta chain, beta 2 precursor
X68194_at	Hs.80919	NM_006754; synaptophysin-like protein isoform a NM_182715; synaptophysin-like protein isoform b
X73882_at	Hs.254605	NM_003980; microtubule-associated protein 7
X78520_at	Hs.372528	NM_001829; chloride channel 3
X95632_s_at	Hs.387906	NM_005759; abl-interactor 2
Z29331_at	Hs.372758	NM_003344; ubiquitin-conjugating enzyme E2H isoform 1 NM_182697; ubiquitin-conjugating enzyme E2H isoform 2
Z48605_at	Hs.421825	NM_006903; inorganic pyrophosphatase 2 isoform 2 NM_176865; NM_176866; inorganic pyrophosphatase 2 isoform 3 NM_176867; inorganic pyrophosphatase 2 isoform 4 NM_176869; inorganic pyrophosphatase 2 isoform 1
Z74615_at	Hs.172928	NM_000088; alpha 1 type I collagen preproprotein

Recurrence predictor

We furthermore tested an outcome predictor able to identify the likely presence or absence of recurrence in patients with superficial Ta tumours (see Table 16).

Table 16. Patient disease course information – recurrence vs. no recurrence

From the hierarchical cluster analysis of the tumour samples we found that the tumours with a high recurrence frequency were separated from the tumours with low recurrence frequency. To study this further we profiled two groups of Ta tumours- 15 tumours with low recurrence frequency and 16 tumours with high recurrence frequency. To avoid influence from other tumour characteristics we only used tumours that showed the same growth pattern and tumours that showed no sign of concomitant carcinoma *in situ*. Furthermore, the tumours were all primary tumours. The tumours used for identifying genes differentially expressed in recurrent and non-recurrent tumours are listed in Table 16 below.

Table 16 Disease course information of all patients involved.

Group	Patient	Tumour (date)	Pattern	Carcinoma *in situ*	Time to recurrence
A	968-1	Ta gr2	Papillary	no	27 month
A	928-1	Ta gr2	Papillary	no	38 month.
A	934-1	Ta gr2 (220798)	Papillary	no	-
A	709-1	Ta gr2 (210798)	Papillary	no	-
A	930-1	Ta gr2 (300698)	Papillary	no	-
A	524-1	Ta gr2 (201095)	Papillary	no	-

A	455-1	Ta gr2 (060695)	Papillary	no	-
A	370-1	Ta gr2 (100195)	Papillary	no	-
A	810-1	Ta gr2 (031097)	Papillary	no	-
A	1146-1	Ta gr2 (231199)	Papillary	no	-
A	1161-1	Ta gr2 (101299)	Mixed	no	-
A	1006-1	Ta gr2 (231198)	Papillary	no	-
A	942-1	Ta gr2	Papillary	no	24 month.
A	1060-1	Ta gr2	Papillary	no	36 month.
A	1255-1	Ta gr2	Papillary	no	24 month.
B	441-1	Ta gr2	Papillary	no	6 month.
B	780-1	Ta gr2	Papillary	no	2 month.
B	815-2	Ta gr2	Papillary	no	6 month.
B	829-1	Ta gr2	Papillary	no	4 month.
B	861-1	Ta gr2	Papillary	no	4 month.
B	925-1	Ta gr2	Papillary	no	5 month.
B	1008-1	Ta gr2	Papillary	no	5 month.
B	1086-1	Ta gr2	Papillary	no	6 month.
B	1105-1	Ta gr2	Papillary	no	8 month.
B	1145-1	Ta gr2	Papillary	no	4 month.
B	1327-1	Ta gr2	Papillary	no	5 month.
B	1352-1	Ta gr2	Papillary	no	6 month.
B	1379-1	Ta gr2	Papillary	no	5 month.
B	533-1	Ta gr2	Papillary	no	4 month.
B	679-1	Ta gr2	Papillary	no	4 month.
B	692-1	Ta gr2	Papillary	no	5 month.

Group A: Primary tumours from patients with no recurrence of the disease for 2 years.
Group B: Primary tumours from patients with recurrence of the disease within 8 months.

Supervised learning prediction of recurrence

In this part of the work we identified genes differentially expressed between non-recurring and recurring tumours. Cross-validation and prediction was performed as previously described, except that genes are selected based on the value of the Wilcoxon statistic for difference between the two groups.

Prediction performance

The prediction performance was tested using from 1-200 genes in the cross-validation loops. Figure 11 shows that the lowest error rate (8 errors) is obtained in e.g. the cross-validation model using from 39 genes. Based on this we selected this cross-validation model as our final predictor. The results of the predictions from the 39 gene cross-validation loops are listed in Table 17. The predictor misclassified four of the samples in each group and in one of the predictions the difference in the distances between the two group means is below the 5% difference limit as described above.

The probability of misclassifying 8 or less arrays by a random classification is 0.0053.

Table 17. Recurrence prediction results of 39 gene cross-validation loops.
Group A: Primary tumours from patients with no recurrence of the disease for 2 years. Group B: Primary tumours from patients with recurrence of the disease within 8 months. Prediction, 0=no recurrence, 1=recurrence.

Group	Patient	Tumour (date)	Prediction	Error	Prediction strength
A	968-1	Ta gr2	0		0.19
A	928-1	Ta gr2	0		0.49
A	934-1	Ta gr2 (220798)	0		1.73
A	709-1	Ta gr2 (210798)	0		0.45
A	930-1	Ta gr2 (300698)	0		0.82
A	524-1	Ta gr2 (201095)	0		0.14
A	455-1	Ta gr2 (060695)	1	*	0.68
A	370-1	Ta gr2 (100195)	0		0.32
A	810-1	Ta gr2 (031097)	0		0.45
A	1146-1	Ta gr2 (231199)	0		0.98
A	1161-1	Ta gr2 (101299)	0		0.03
A	1006-1	Ta gr2 (231198)	1	*	1.57
A	942-1	Ta gr2	0		0.31
A	1060-1	Ta gr2	1	*	0.81
A	1255-1	Ta gr2	1	*	0.71
B	441-1	Ta gr2	1		1.03
B	780-1	Ta gr2	1		0.37
B	815-2	Ta gr2	1		0.35
B	829-1	Ta gr2	1		0.75
B	861-1	Ta gr2	0	*	2.55
B	925-1	Ta gr2	1		0.78
B	1008-1	Ta gr2	0	*	0.12
B	1086-1	Ta gr2	0	*	0.51
B	1105-1	Ta gr2	1		0.37
B	1145-1	Ta gr2	1		0.44
B	1327-1	Ta gr2	1		1.96
B	1352-1	Ta gr2	0	*	0.97
B	1379-1	Ta gr2	1		0.67
B	533-1	Ta gr2	1		0.31
B	679-1	Ta gr2	1		0.82
B	692-1	Ta gr2	1		0.45

The optimal number of genes in cross-validation loops was found to be 39 (75% of the samples were correct classified, p<0.006) and from this we selected those 26 genes that were used in at least 75% of the cross-validation loops to constitute our final recurrence predictor.

Consequently, this set of genes is to be used for predicting recurrence in independent samples. We tested the strength of the predictive genes by permutation analysis, see Table 18. We selected the genes used in at least 29 of the 31 cross-validation loops to constitute our final recurrence prediction model. The expression pattern of those 26 genes is shown in fig. 12.

Table 18. The 26 genes that we find optimal for recurrence prediction.

Feature	Unigene build 168	Description	Number	Test (W-N)
AF006041_at	Hs.336916	NM_001350; death-associated protein 6	31	0.054 (161-7)
D21337_at	Hs.408	NM_001847; type IV alpha 6 collagen isoform A precursor NM_033641; type IV alpha 6 collagen isoform B precursor	31	0.058 (160-6)
D49387_at	Hs.294584	NM_012212; NADP-dependent leukotriene B4 12-hydroxydehydrogenase	31	0.118 (313-8)
D64154_at	Hs.90107	NM_007002; adhesion regulating molecule 1 precursor NM_175573; adhesion regulating molecule 1 precursor	31	0.078 (165-9)
D83780_at	Hs.437991	NM_014846; KIAA0196 gene product	31	0.094 (159-4)
D87258_at	Hs.75111	NM_002775; protease, serine, 11	30	0.112 (168-11
D87437_at	Hs.43660	NM_014837; chromosome 1 open reading frame 16	31	0.058 (160-6)
HG1879-HT1919_at			31	0.122 (314-7)
HG3076-HT3238_s_at			31	0.080 (309-17
HG511-HT511_at			31	0.348 (319-2)
L34155_at	Hs.83450	NM_000227; laminin alpha 3 subunit precursor	31	0.122 (314-7)
L38928_at	Hs.118131	NM_006441; 5,10-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)	29	0.348 (319-2)
L49169_at	Hs.75678	NM_006732; FBJ murine osteosarcoma viral oncogene homolog B	31	0.108 (155-2)
M16938_s_at	Hs.820	NM_004503; homeo box C6 isoform 1 NM_153693; homeo box C6 isoform 2	29	0.09 (170-16)
M63175_at	Hs.295137	NM_001144; autocrine motility factor receptor isoform a NM_138958; autocrine motility factor receptor isoform b	29	0.098 (308-18
M64572_at	Hs.405666	NM_002829; protein tyrosine phosphatase, non-receptor type 3	31	0.064 (305-31
M98528_at	Hs.79404	NM_014392; DNA segment on chromosome 4 (unique) 234 expressed sequence	31	0.122 (314-7)
U21858_at	Hs.60679	NM_003187; TBP-associated factor 9 NM_016283; adrenal gland protein AD-004	31	0.122 (314-7)
U45973_at	Hs.178347	NM_016532; skeletal muscle and kidney enriched inositol phosphatase isoform 1 NM_130766; skeletal muscle and kidney enriched inositol phosphatase isoform 2	31	0.094 (310-14
U58516_at	Hs.3745	NM_005928; milk fat globule-EGF factor 8 protein	29	0.100 (175-28
U62015_at	Hs.8867	NM_001554; cysteine-rich, angiogenic inducer, 61	31	0.106 (169-13
U66702_at	Hs.74624	NM_002847; protein tyrosine phosphatase, receptor type, N polypeptide 2 isoform 1 precursor NM_130842; protein	31	0.146 (149-1)

		tyrosine phosphatase, receptor type, N polypeptide 2 isoform 2 precursor NM_130843; protein tyrosine phosphatase, receptor type, N polypeptide 2 isoform 3 precursor		
U70439_s_at	Hs.84264	NM_006401; acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	30	0.08 (309-17)
U94855_at	Hs.381255	NM_003754; eukaryotic translation initiation factor 3, subunit 5 epsilon, 47kDa	30	0.092 (311-12)
X63469_at	Hs.77100	NM_002095; general transcription factor IIE, polypeptide 2, beta 34kDa	31	0.092 (311-12)
Z23064_at	Hs.380118	NM_002139; RNA binding motif protein, X chromosome	30	0.066 (307-24)

Number: Number of times the gene has been used in a cross-validation loop. Test: The numbers in parenthesis are the value W of the Wilcoxon test statistic for no difference between the two groups together with the number N of genes for which the Wilcoxon test statistic is bigger than or equal to the value W. The test value is obtained from 500 permutations of the arrays. In each permutation we form new pseudogroups where both of the pseudogroups have the same proportion of arrays from the two original groups. For each permutation we count the number of genes for which the Wilcoxon test statistic based on the pseudogroups is bigger than or equal to W, and the test value is the proportion of the permutations for which this number is bigger than or equal to N. Thus the test value measures the significance of the observed value W. Consequently, for most of our selected genes we only find as least as good predictive genes in about 10% of the formed pseudogroups.

We present data on expression patterns that classify the benign and muscle-invasive bladder carcinomas. Furthermore, we can identify subgroups of bladder cancer such as Ta tumours with surrounding CIS, Ta tumours with a high probability of progression as well as recurrence, and T2 tumours with squamous metaplasia. As a novel finding, the matrix remodelling gene cluster was specifically expressed in the tumours having the worst prognosis, namely the T2 tumours and tumours surrounded by CIS. For some of these genes new small molecule inhibitors already exist (Kerr et al. 2002), and thus they form drug targets. At present it is not possible clinically to identify patients who will experience recurrence and not recurrenc, but it would be a great benefit to both the patients and the health system by reducing the number of unnecessary control examinations in bladder tumour patients. To determine the optimal gene-set for separating non-recurrent and recurrent tumours, we again applied a cross-validation scheme using from 1-200 genes. We determined the optimal number of genes in cross-validation loops to be 39 (75% of the samples were correct classified, $p<0.01$, Figure 11) and from this we selected those 26 genes (Figure 12) that were used in at least 75% of the cross-validation loops to constitute our final recurrence predictor. Consequently, this set of genes is to be used for predicting recurrence in independent sam-

ples. We tested the strength of the predictive genes by performing 500 permutations of the arrays. This revealed that for most of our predictive genes we would only in a small number of the new pseudo-groups obtain at least as good predictors as in the real groups.

Biological material

66 bladder tumour biopsies were sampled from patients following removal of the necessary amount of tissue for routine pathology examination. The tumours were frozen immediately after surgery and stored at -80°C in a guanidinium thiocyanate solution. All tumours were graded according to Bergkvist *et al.* 1965 and re-evaluated by a single pathologist. As normal urothelial reference samples we used a pool of biopsies (from 37 patients) as well as three single bladder biopsies from patients with prostatic hyperplasia or urinary incontinence. Informed consent was obtained in all cases and protocols were approved by the local scientific ethical committee.

RNA purification and cRNA preparation

Total RNA was isolated from crude tumour biopsies using a Polytron homogenisator and the RNAzol B RNA isolation method (WAK-Chemie Medical GmbH). 10 μg total RNA was used as starting material for the cDNA preparation. The first and second strand cDNA synthesis was performed using the SuperScript Choice System (Life Technologies) according to the manufacturers instructions except using an oligo-dT primer containing a T7 RNA polymerase promoter site. Labelled cRNA was prepared using the BioArray High Yield RNA Transcript Labelling Kit (Enzo). Biotin labelled CTP and UTP (Enzo) were used in the reaction together with unlabeled NTP's. Following the IVT reaction, the unincorporated nucleotides were removed using RNeasy columns (Qiagen).

Array hybridisation and scanning

15 μg of cRNA was fragmented at 94^0C for 35 min in a fragmentation buffer containing 40 mM Tris-acetate pH 8.1, 100 mM KOAc, 30 mM MgOAc. Prior to hybridisation, the fragmented cRNA in a 6xSSPE-T hybridisation buffer (1 M NaCl, 10 mM Tris pH 7.6, 0.005% Triton), was heated to 95^0C for 5 min and subsequently to 45^0C for 5 min before loading onto the Affymetrix probe array cartridge (HuGeneFL). The probe array was then incubated for 16 h at 45^0C at constant rotation (60 rpm). The washing and staining procedure was performed in the Affymetrix Fluidics Station. The probe array was exposed to 10 washes in 6xSSPE-T at 25^0C followed by 4 washes in 0.5xSSPE-T at 50^0C. The biotinylated cRNA was stained with a streptavidin-phycoerythrin conjugate, final concentration 2 μg/μl (Molecular Probes, Eugene, OR) in 6xSSPE-T for 30 min at 25^0C followed by 10 washes in 6xSSPE-T at 25^0C. The probe arrays were scanned at 560 nm using a confocal laser-scanning microscope (Hewlett Packard GeneArray Scanner G2500A). The readings from the quantitative scanning were analysed by the Affymetrix Gene Expression Analysis Software. An antibody amplifica-

tion step followed using normal goat IgG as blocking reagent, final concentration 0.1 mg/ml (Sigma) and biotinylated anti-streptavidin antibody (goat), final concentration 3 μg/ml (Vector Laboratories). This was followed by a staining step with a streptavidin-phycoerythrin conjugate, final concentration 2 μg/μl (Molecular Probes, Eugene, OR) in 6xSSPE-T for 30 min at 25^{0}C and 10 washes in 6xSSPE-T at 25^{0}C. The arrays were then subjected to a second scan under similar conditions as described above.

Class discovery using hierarchical clustering

All microarray results were scaled to a global intensity of 150 units using the Affymetrix GeneChip software. Other ways of array normalisation exist (Li and Hung 2001), however, using the dCHIP approach did not change the expression profiles of the obtained classifier genes in this study (results not shown). For hierarchical cluster analysis and molecular classification procedures we used expression level ratios between tumours and the normal urothelium reference pool calculated using the comparison analysis implemented in the Affymetrix GeneChip software. In order to avoid expression ratios based on saturated gene-probes, we used the antibody amplified expression-data for genes with a mean Average Difference value across all samples below 1000 and the non-amplified expression-data for genes with values equal to or above 1000 in mean Average Difference value across all samples. Consequently, gene expression levels across all samples were either from the amplified or the non-amplified expression-data. We applied different filtering criteria to the expression data in order to avoid including non-varying and very low expressed genes in the data analysis. Firstly, we selected only genes that showed significant changes in expression levels compared to the normal reference pool in at least three samples. Secondly, only genes with at least three "Present" calls across all samples were selected. Thirdly, we eliminated genes varying less than 2 standard deviations across all samples. The final gene-set contained 1767 genes following filtering. Two-way hierarchical agglomerative cluster analysis was performed using the Cluster software[25]. We used average linkage clustering with a modified Pearson correlation as similarity metric. Genes and arrays were median centred and normalised to the magnitude of 1 prior to cluster analysis. The TreeView software was used for visualisation of the cluster analysis results (Eisen et al. 1998). Multidimensional scaling was performed on median centred and normalised data using an implementation in the SPSS statistical software package.

Tumour stage classifier

We based the classifier on the log-transformed expression level ratios. For these transformed values we used a normal distribution with the mean dependent on the gene and the group (Ta, T1, and T2, respectively) and the variance dependent on the gene only. For each gene we calculated the variation within the groups (W) and the three variations between two groups (B(Ta/T1), B(Ta/T2), B(T1/T2)) and used the three ratios B/W to select genes. We

selected those genes having a high value of B(Ta/T1)/W, those genes having a high value of B(Ta/T2)/W, and those genes with a high value of B(T1/T2)/W. To classify a sample, we calculated the sum over the genes of the squared distance from the sample value to the group mean, standardised by the variance. Thus, we got a distance to each of the three groups and the sample was classified as belonging to the group in which the distance was smallest. When calculating these distances the group means and the variances were estimated from all the samples in the training set excluding the sample being classified.

Recurrence prediction using a supervised learning method

Average Difference values were generated using the Affymetrix GeneChip software and all values below 20 were set to 20 to avoid very low and negative numbers. We only included genes that had a "Present" call in at least 7 samples and genes that showed intensity variation (Max-Min>100, Max/Min>2). The values were log transformed and rescaled. We used a supervised learning method essentially as described (Shipp et al. 2002). Genes were selected using t-test statistics and cross-validation and sample classification was performed as described above.

Immunohistochemistry

Tumour tissue microarrays were prepared essentially as described (Kononen et al. 1998), with four representative 0.6 mm paraffin cores from each study case. Immunohistochemical staining was performed using standard highly sensitive techniques after appropriate heat-induced antigen retrieval. Primary polyclonal goat antibodies against Smad 6 (S-20) and cyclin G2 (N-19) were from Santa Cruz Biotechnology. Antibodies to p53 (monoclonal DO-7) and Her-2 (polyclonal anti-c-erbB-2) were from Dako A/S. Ki-67 monoclonal antibody (MIBI) was from Novocastra Laboratories Ltd. Staining intensity was scored at four levels, Negative, Weak, Moderate and Strong by an experienced pathologist who considered both colour intensity and number of stained cells, and who was unaware of array results.

EXAMPLE 3

A molecular classifier detects carcinoma in situ expression signatures in tumors and normal urothelium of the bladder.

Clinical samples

Bladder tumour samples were obtained directly from surgery following removal of tissue for routine pathological examination. The samples were immediately submerged in a guadinium thiocyanate solution for RNA preservation and stored at –80° C. Informed consent was obtained in all cases, and the protocols were approved by the scientific ethical committee of Aarhus County. Samples in the No-CIS group were selected based on the following criteria: a) Ta tumours with no CIS in selected site biopsies in all visits; b) no previous muscle invasive tumour. Samples in the CIS group were selected based on the criteria: a) Ta or T1

tumours with CIS in selected site biopsies in any visit (preferable Ta tumours with CIS in the sampling visit); b) no previous muscle invasive tumours. Normal biopsies were obtained from individuals with prostatic hyperplasia or urinary incontinence. CIS and "normal" biopsies were obtained from cystectomy specimens directly following removal of the bladder. A grid was placed in the bladder for orientation and biopsies were taken from 8 positions covering the bladder surface. At each position, three biopsies were taken - two for pathologic examination and one in between these for RNA extraction for microarray expression profiling. The samples for RNA extraction were immediately transferred to the guadinium thiocyanate solution and stored at –80° C until use. Samples used for RNA extraction were assumed to have CIS if CIS was detected in both adjacent biopsies. The "normal" samples were assumed to be normal if both adjacent biopsies were normal.

cRNA preparation, array hybridisation and scanning

Purification of total RNA, preparation of cRNA from cDNA and hybridisation and scanning were performed as previously described (Dyrskjot et al. 2003). The labelled samples were hybridised to Affymetrix U133A GeneChips.

Expression data analysis

Following scanning all data were normalised using the RMA normalisation approach in the Bioconductor Affy package to R. Variation filters were applied to the data to eliminate non-varying and presumably non-expressed genes. For gene-set 1 this was done by only including genes with a minimum expression above 200 in at least 5 samples and genes with max/min expression intensities above or equal to 3. The filtering for gene-set 2 including only genes with a minimum expression of 200 in at least 3 samples and genes with max/min expression intensities above or equal to 3. Average linkage hierarchical cluster analysis was carried out using the Cluster software with a modified Pearson correlation as similarity metric (Eisen et al. 1998). We used the TreeView software for visualisation of the cluster analysis results (Eisen et al. 1998). Genes were log-transformed, median centred and normalised to the magnitude of 1 before clustering. We used GeneCluster 2.0 (http://www-genome.wi.mit.edu/cancer/software/genecluster2/gc2.html) for the supervised selection of markers and for permutation testing. The algorithms used in the software are based on (Golub et al. 1999, Tamayo et al. 1999). Classifiers for CIS detection were built using the same methods as described previously (Dyrskjot et al. 2003).

Gene expression profiling

We used high-density oligonucleotide microarrays for gene expression profiling of approximately 22,000 genes in 28 superficial bladder tumour biopsies (13 tumours with surrounding CIS and 15 without surrounding CIS) and in 13 invasive carcinomas. See table 19 for patient disease course descriptions. Furthermore, expression profiles were obtained

from 9 normal biopsies and from 10 biopsies from cystectomy specimens (5 histologically normal biopsies and 5 biopsies with CIS).

Table 19 *Clinical data on patient disease courses and results of molecular CIS classification*

Sample group [a]	Patient [b]	Previous tumours	Tumour analysed	Subsequent tumours	CIS [c]	CIS classifier [d]
1	1060-1		Ta gr2	2 Ta	No	No CIS
1	1146-1		Ta gr2		No	No CIS
1	1216-1		Ta gr2		No	No CIS
1	1303-1		Ta gr2		No	No CIS
1	524-1		Ta gr2		No	No CIS
1	692-1		Ta gr2	2 Ta	No	No CIS
1	1264-1		Ta gr3	20 Ta	No	No CIS
1	1350-1		Ta gr3	1 Ta	No	No CIS
1	1354-1		Ta gr3	11 T1	No	No CIS
1	775-1		Ta gr3	1 Ta	No	No CIS
1	1066-1		Ta gr3	1 Ta	No	No CIS
1	1276-1		Ta gr3	2 T1	No	No CIS
1	1070-1		Ta gr3	1 Ta	No	No CIS
1	989-1		Ta gr3		No	No CIS
1	1482-1		Ta gr3	20 Ta	No	CIS
2	1345-2	1 T1	Ta gr3		Sampling visit	CIS
2	1062-2		Ta gr3	1 T1	Sampling visit	CIS
2	956-2		Ta gr3	1 Ta	Sampling visit	CIS
2	320-7	1 Ta, 2 T1	Ta gr3	2 Ta	Sampling visit	CIS
2	1330-1		Ta gr3		Sampling visit	CIS
2	602-8	5 Ta	Ta gr3	3 Ta	Sampling visit	CIS
2	763-1		Ta gr2	14 Ta	Sampling visit	CIS
2	1024-1		T1 gr3	2 Ta, 1 T1	Sampling visit	CIS
2	1182-1		Ta gr3	7 Ta	Subsequent visit	CIS
2	1093-1		Ta gr3	4 Ta, 1 T1	Subsequent visit	CIS
2	979-1		Ta gr3		Sampling visit	CIS
2	1337-1		T1 gr3		Sampling visit	CIS
2	1625-1		Ta gr2		Sampling visit	CIS
3	1015-1		T3b gr4		No	-
3	1337-1		T4a gr3		Sampling visit	-
3	1041-1		T4b gr3		No	-
3	1044-1		T4b gr3		ND	-
3	1055-1	1 Ta gr2	T3a gr3		No	-
3	1109-1		T2 gr3	1 T2-4	No	-
3	1124-1		T4a gr3	2 T2-4	No	-
3	1154-1		T3a gr3	1 Ta, 1 T2-4	No	-
3	1167-1	1 T2-4	T3b gr4	2 T2-4	ND	-
3	1178-1		T4b gr3		ND	-
3	1215-1		T4b gr3		ND	-
3	1271-1		T3b gr4		No	-
3	1321-1	1 T1	T3b gr?		ND	-

[a] The tumour groups involved were TCC without CIS (1), TCC with CIS (2) and invasive TCC (3).

[b] The numbers indicate the patient number followed by the clinic visit number.

[c] CIS in selected site biopsies in previous, present or subsequent visits to the clinic. ND: not determined.

[d] Molecular classification of the samples using 25 genes in cross-validation loops.

Hierarchical cluster analysis

Following appropriate normalisation and expression intensity calculations we selected those genes that showed high variation across the 41 TCC samples for further analysis. The filtering produced a gene-set consisting of 5,491 genes (gene-set 1) and two-way hierarchical cluster analysis was performed based on this gene-set. The sample clustering showed a separation of the three groups of samples with only few exceptions (Fig. 14a). Superficial TCC with surrounding CIS clustered in the one main branch of the dendrogram, while the superficial TCC without CIS and the invasive TCC clustered in two separate sub-branches in the other main branch of the dendrogram. The only exceptions were that the invasive TCC samples 1044-1 and 1124-1 clustered in the CIS group and two TCC with CIS clustered in the invasive group (samples 1330-1 and 956-2). The only TCC without CIS that clustered in the CIS group was sample 1482-1. The distinct clustering of the tumour groups indicated a large difference in gene expression patterns.

Hierarchical clustering of the genes (Fig. 14c) identified large clusters of genes characteristic for the each tumour phenotype. Cluster 1 showed a cluster of genes down-regulated in cystectomy biopsies, TCC with adjacent CIS and in some invasive carcinomas (Fig. 14c). There is no obvious functional relationship between the genes in this cluster. Cluster 2 showed a tight cluster of genes related to immunology and cluster 3 contained mostly genes expressed in muscle and connective tissue. Expression of genes in this cluster was observed in the normal and cystectomy samples, in a fraction of the TCC with CIS and in the invasive tumours. Cluster 4 contained genes up-regulated in the cystectomy biopsies, TCC with adjacent CIS and in invasive carcinomas (Fig. 14c). This cluster includes genes involved in cell cycle regulation, cell proliferation and apoptosis. However, for most of the genes in this cluster there is not apparent functional relationship either. Comparisons of chromosomal location of the genes in the clusters revealed no correlation between the observed gene clusters and chromosomal position of the identified genes. A positive correlation could have indicated chromosomal loss or gain or chromosomal inactivation by e.g. methylation of common promoter regions.

To analyse the impact of surrounding CIS lesions further we used the 28 superficial tumours only, and created a new gene set consisting of 5,252 varying genes (gene-set 2). Hierarchical cluster analysis of the tumour samples (Figure 13b) based on the new gene-set separated the samples according to the presence of CIS in the surrounding urothelium with

only 1 exception (P< 0.000001, χ^2-test). Sample 1482-1 clustered in the TCC with CIS group, however, no CIS has been detected in selected site biopsies during routine examinations of this patient. Tumour samples 1182-1 and 1093-1 did not have CIS in selected site biopsies in the same visit as the profiled tumour but showed this in later visits. However, the profile of these two superficial tumour samples already showed the adjacent CIS profile.

Marker selection

To delineate the tumours with surrounding CIS from the tumours without CIS we used t-test statistics to select the 50 most up-regulated genes in each group (Figure 15a). Permutation of the sample labels 500 times revealed that the 50 genes up-regulated in the CIS-group are highly significant differentially expressed and unlikely to find by chance, as all markers were significant on a 5% confidence level. Consequently, in 500 random datasets it was only possible to select as good genes in less than 5% of the datasets. The 50 genes up-regulated in the no-CIS group showed a poorer performance in the permutation tests, as these were not significant on a 5% confidence level. See Table 20 for details. The relative expression of these 100 genes is 9 normal and 10 biopsies from cystectomies with CIS are shown in figure 15b. The no-CIS profile was found in all of the normal samples. However, all histologically normal samples adjacent to the CIS lesions as well as the CIS biopsies showed the CIS profile.

Table 20.The best 100 markers

Feature (U133 array)	Class	T-test	Perm 1%	Perm 5%	Perm 10%	UniGene Build 162	RefSeq;description
221204_s_at	no_CIS	3.74	5.12	4.61	4.33	Hs.326444	NM_018058; cartilage acidic protein 1
205927_s_at	no_CIS	3.67	4.53	3.98	3.73	Hs.1355	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
210143_at	no_CIS	3.35	4.03	3.73	3.45	Hs.188401	NM_007193; annexin A10
204540_at	no_CIS	3.15	3.87	3.51	3.32	Hs.433839	NM_001958; eukaryotic translation elongation factor 1 alpha 2
214599_at	no_CIS	3.02	3.75	3.37	3.14	Hs.157091	NM_005547; involucrin
203649_s_at	no_CIS	2.84	3.63	3.20	3.00	Hs.76422	NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)
203980_at	no_CIS	2.74	3.47	3.12	2.89	Hs.391561	NM_001442; fatty acid binding protein 4, adipocyte
209270_at	no_CIS	2.39	3.38	3.10	2.85	Hs.436983	NM_000228; laminin subunit beta 3 precursor
206658_at	no_CIS	2.35	3.37	3.05	2.78	Hs.284211	NM_030570; uroplakin 3B iso-

							form a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b
220779_at	no_CIS	2.35	3.33	2.97	2.73	Hs.149195	NM_016233; peptidylarginine deiminase type III
216971_s_at	no_CIS	2.28	3.29	2.91	2.71	Hs.79706	NM_000445; plectin 1, intermediate filament binding protein 500kDa
206191_at	no_CIS	2.25	3.24	2.86	2.68	Hs.47042	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3
218484_at	no_CIS	2.18	3.20	2.81	2.62	Hs.221447	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog
221854_at	no_CIS	2.1	3.19	2.80	2.60	Hs.313068	NM_000299; plakophilin 1
203792_x_at	no_CIS	2.02	3.16	2.74	2.55	Hs.371617	NM_007144; ring finger protein 110
207862_at	no_CIS	2.01	3.16	2.72	2.52	Hs.379613	NM_006760; uroplakin 2
218960_at	no_CIS	1.93	3.14	2.65	2.47	Hs.414005	NM_019894; transmembrane protease, serine 4 isoform 1 NM_183247; transmembrane protease, serine 4 isoform 2
203009_at	no_CIS	1.93	3.12	2.62	2.45	Hs.155048	NM_005581; Lutheran blood group (Auberger b antigen included)
204508_s_at	no_CIS	1.88	3.10	2.60	2.42	Hs.279916	NM_017689; hypothetical protein FLJ20151
211692_s_at	no_CIS	1.87	3.06	2.58	2.39	Hs.87246	NM_014417; BCL2 binding component 3
206465_at	no_CIS	1.86	3.04	2.54	2.38	Hs.277543	NM_015162; lipidosin
206122_at	no_CIS	1.85	2.92	2.52	2.36	Hs.95582	NM_006942; SRY-box 15
206393_at	no_CIS	1.83	2.89	2.49	2.33	Hs.83760	NM_003282; troponin I, skeletal, fast
214639_s_at	no_CIS	1.79	2.87	2.49	2.30	Hs.67397	NM_005522; homeobox A1 protein isoform a NM_153620; homeobox A1 protein isoform b
214630_at	no_CIS	1.79	2.84	2.44	2.28	Hs.184927	NM_000497; cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 precursor
204465_s_at	no_CIS	1.77	2.81	2.42	2.27	Hs.76888	NM_004692; NM_032727; internexin neuronal intermediate filament protein, alpha
204990_s_at	no_CIS	1.76	2.79	2.41	2.24	Hs.85266	NM_000213; integrin, beta 4
205453_at	no_CIS	1.75	2.77	2.39	2.22	Hs.290432	NM_002145; homeo box B2
215812_s_at	no_CIS	1.74	2.77	2.37	2.20	Hs.499113	NM_018058; cartilage acidic protein 1

217040_x_at	no_CIS	1.74	2.75	2.36	2.18	Hs.95582	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
203759_at	no_CIS	1.73	2.75	2.34	2.17	Hs.75268	NM_007193; annexin A10
211002_s_at	no_CIS	1.73	2.74	2.33	2.17	Hs.82237	NM_001958; eukaryotic translation elongation factor 1 alpha 2
216641_s_at	no_CIS	1.73	2.73	2.31	2.15	Hs.18141	NM_005547; involucrin
221660_at	no_CIS	1.71	2.67	2.30	2.13	Hs.247831	NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)
220026_at	no_CIS	1.71	2.66	2.28	2.13	Hs.227059	NM_001442; fatty acid binding protein 4, adipocyte
209591_s_at	no_CIS	1.69	2.63	2.28	2.11	Hs.170195	NM_000228; laminin subunit beta 3 precursor
219922_s_at	no_CIS	1.68	2.61	2.26	2.08	Hs.289019	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b
201641_at	no_CIS	1.67	2.61	2.26	2.07	Hs.118110	NM_016233; peptidylarginine deiminase type III
204952_at	no_CIS	1.66	2.59	2.24	2.07	Hs.377028	NM_000445; plectin 1, intermediate filament binding protein 500kDa
204487_s_at	no_CIS	1.65	2.59	2.23	2.06	Hs.367809	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3
210761_s_at	no_CIS	1.64	2.59	2.23	2.05	Hs.86859	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog
217626_at	no_CIS	1.63	2.58	2.21	2.04	Hs.201967	NM_000299; plakophilin 1
204380_s_at	no_CIS	1.62	2.58	2.19	2.03	Hs.1420	NM_007144; ring finger protein 110
205455_at	no_CIS	1.61	2.58	2.17	2.02	Hs.2942	NM_006760; uroplakin 2
205073_at	no_CIS	1.61	2.58	2.17	2.01	Hs.152096	NM_019894; transmembrane protease, serine 4 isoform 1 NM_183247; transmembrane protease, serine 4 isoform 2
203287_at	no_CIS	1.61	2.58	2.16	2.00	Hs.18141	NM_005581; Lutheran blood group (Auberger b antigen included)
210735_s_at	no_CIS	1.58	2.55	2.15	1.99	Hs.5338	NM_017689; hypothetical protein FLJ20151
203842_s_at	no_CIS	1.57	2.54	2.15	1.97	Hs.172740	NM_014417; BCL2 binding component 3
206561_s_at	no_CIS	1.57	2.53	2.14	1.96	Hs.116724	NM_015162; lipidosin
214752_x_at	no_CIS	1.56	2.52	2.13	1.95	Hs.195464	NM_006942; SRY-box 15

217028_at	CIS	4.87	5.17	4.67	4.40	Hs.421986	NM_003282; troponin I, skeletal, fast
213975_s_at	CIS	4.65	4.43	4.01	3.76	Hs.234734	NM_005522; homeobox A1 protein isoform a NM_153620; homeobox A1 protein isoform b
201859_at	CIS	4.59	4.15	3.70	3.45	Hs.1908	NM_000497; cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 precursor
219410_at	CIS	4.49	3.98	3.49	3.29	Hs.104800	NM_004692; NM_032727; internexin neuronal intermediate filament protein, alpha
207173_x_at	CIS	4.37	3.88	3.33	3.11	Hs.443435	NM_000213; integrin, beta 4
214651_s_at	CIS	4.14	3.83	3.22	2.99	Hs.127428	NM_002145; homeo box B2
201858_s_at	CIS	4.06	3.78	3.09	2.91	Hs.1908	NM_018058; cartilage acidic protein 1
211430_s_at	CIS	4.03	3.63	3.05	2.83	Hs.413826	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
213891_s_at	CIS	3.86	3.63	3.02	2.77	Hs.359289	NM_007193; annexin A10
221872_at	CIS	3.82	3.52	2.89	2.73	Hs.82547	NM_001958; eukaryotic translation elongation factor 1 alpha 2
212386_at	CIS	3.77	3.50	2.87	2.69	Hs.359289	NM_005547; involucrin
211161_s_at	CIS	3.76	3.42	2.84	2.65		NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)
214669_x_at	CIS	3.55	3.36	2.80	2.62	Hs.377975	NM_001442; fatty acid binding protein 4, adipocyte
217388_s_at	CIS	3.44	3.31	2.79	2.58	Hs.444471	NM_000228; laminin subunit beta 3 precursor
203477_at	CIS	3.36	3.28	2.75	2.56	Hs.409034	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b
204688_at	CIS	3.35	3.26	2.74	2.52	Hs.409798	NM_016233; peptidylarginine deiminase type III
218718_at	CIS	3.35	3.22	2.70	2.48	Hs.43080	NM_000445; plectin 1, intermediate filament binding protein 500kDa
215176_x_at	CIS	3.32	3.14	2.67	2.45	Hs.503443	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3
201842_s_at	CIS	3.31	3.11	2.65	2.44	Hs.76224	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog
212667_at	CIS	3.3	3.11	2.63	2.42	Hs.111779	NM_000299; plakophilin 1

209340_at	CIS	3.27	3.10	2.61	2.39	Hs.21293	NM_007144; ring finger protein 110
215379_x_at	CIS	3.26	3.10	2.59	2.39	Hs.449601	NM_006760; uroplakin 2
200762_at	CIS	3.25	3.05	2.56	2.34	Hs.173381	NM_019894; transmembrane protease, serine 4 isoform 1 NM_183247; transmembrane protease, serine 4 isoform 2
211896_s_at	CIS	3.21	3.05	2.53	2.32	Hs.156316	NM_005581; Lutheran blood group (Auberger b antigen included)
204141_at	CIS	3.19	3.05	2.53	2.28	Hs.300701	NM_017689; hypothetical protein FLJ20151
201744_s_at	CIS	3.18	3.03	2.50	2.27	Hs.406475	NM_014417; BCL2 binding component 3
209138_x_at	CIS	3.17	3.03	2.47	2.24	Hs.505407	NM_015162; lipidosin
214677_x_at	CIS	3.14	3.02	2.47	2.23	Hs.449601	NM_006942; SRY-box 15
212077_at	CIS	3.11	2.99	2.46	2.21	Hs.443811	NM_003282; troponin I, skeletal, fast
206392_s_at	CIS	3.11	2.98	2.43	2.20	Hs.82547	NM_005522; homeobox A1 protein isoform a NM_153620; homeobox A1 protein isoform b
212998_x_at	CIS	3.09	2.94	2.40	2.19	Hs.375115	NM_000497; cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 precursor
201616_s_at	CIS	3.08	2.93	2.38	2.18	Hs.443811	NM_004692; NM_032727; internexin neuronal intermediate filament protein, alpha
205382_s_at	CIS	3.07	2.88	2.37	2.15	Hs.155597	NM_000213; integrin, beta 4
212671_s_at	CIS	3.07	2.85	2.35	2.14	Hs.387679	NM_002145; homeo box B2
215121_x_at	CIS	3.06	2.84	2.34	2.13	Hs.356861	NM_018058; cartilage acidic protein 1
200600_at	CIS	3.05	2.83	2.33	2.11	Hs.170328	NM_001910; cathepsin E isoform a preproprotein NM_148964; cathepsin E isoform b preproprotein
202746_at	CIS	3.03	2.80	2.32	2.10	Hs.17109	NM_007193; annexin A10
202917_s_at	CIS	3	2.79	2.31	2.08	Hs.416073	NM_001958; eukaryotic translation elongation factor 1 alpha 2
201560_at	CIS	3	2.79	2.30	2.08	Hs.25035	NM_005547; involucrin
218918_at	CIS	2.99	2.77	2.29	2.06	Hs.8910	NM_000300; phospholipase A2, group IIA (platelets, synovial fluid)
218656_s_at	CIS	2.99	2.76	2.27	2.06	Hs.93765	NM_001442; fatty acid binding protein 4, adipocyte
201088_at	CIS	2.99	2.76	2.26	2.04	Hs.159557	NM_000228; laminin subunit beta 3 precursor

201291_s_at	CIS	2.97	2.75	2.25	2.04	Hs.156346	NM_030570; uroplakin 3B isoform a NM_182683; uroplakin 3B isoform c NM_182684; uroplakin 3B isoform b
215076_s_at	CIS	2.95	2.72	2.24	2.03	Hs.443625	NM_016233; peptidylarginine deiminase type III
212195_at	CIS	2.94	2.71	2.22	2.02	Hs.71968	NM_000445; plectin 1, intermediate filament binding protein 500kDa
209732_at	CIS	2.94	2.68	2.22	2.00	Hs.85201	NM_001248; ectonucleoside triphosphate diphosphohydrolase 3
212192_at	CIS	2.94	2.67	2.22	1.99	Hs.109438	NM_020142; NADH:ubiquinone oxidoreductase MLRQ subunit homolog
221671_x_at	CIS	2.92	2.67	2.20	1.98	Hs.377975	NM_000299; plakophilin 1
211671_s_at	CIS	2.91	2.66	2.20	1.98	Hs.126608	NM_007144; ring finger protein 110
214352_s_at	CIS	2.88	2.66	2.19	1.97	Hs.412107	NM_006760; uroplakin 2

Feature: Probe-set on U133A GeneChip
Class: The group in which the marker is up-regulated
T-test: The t-test value
Perm 1%: The 1% permutation level
Perm 5%: The 5% permutation level
Perm 10%: The 10% permutation level

Construction of a molecular CIS classifier

A classifier able to diagnose CIS from gene expressions in TCC or in bladder biopsies may increase the detection rate of CIS. Our first approach was to be able to classify superficial TCC with or without CIS in the surrounding mucosa. This could have the diverse effect that the number of random biopsies to be taken could be reduced.

We build a CIS-classifier as previously described (Dyrskjot et al. 2003) using cross-validation for determining the optimal number of genes for classifying CIS with fewest errors. The best classifier performance (1 error) was obtained in cross-validation loops using 25 genes (see figure 16); 16 of these were included in 70% of the cross-validation loops and these were selected to represent our final classifier for CIS diagnosis (Fig. 17a and table 21). Permutation analysis shoved that 13 of these were significant at a 1% confidence level – the remaining three genes were above a 10% confidence level.

Table 21. The 16 gene molecular classifier of CIS

Feature (U133a	Class	t-test	Perm 1%	Perm 5%	Perm 10%	UniGene Build 162	RefSeq;description

array)							
213633_at	no_CIS	1.51	2.46	2.04	1.85	Hs.97858	NM_018957; SH3-domain binding protein 1
212784_at	no_CIS	1.36	2.27	1.86	1.70	Hs.388236	NM_015125; capicua homolog
209241_x_at	no_CIS	1.13	1.78	1.48	1.33	Hs.112028	NM_015716; mis-shapen/NIK-related kinase isoform 1 NM_153827; mis-shapen/NIK-related kinase isoform 3 NM_170663; mis-shapen/NIK-related kinase isoform 2
217941_s_at	CIS	2.3	1.96	1.66	1.47	Hs.8117	NM_018695; erbb2 interacting protein
201877_s_at	CIS	2.27	1.90	1.62	1.45	Hs.249955	NM_002719; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform a NM_178586; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform b NM_178587; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform c NM_178588; gamma isoform of regulatory subunit B56, protein phosphatase 2A isoform d
209630_s_at	CIS	1.97	1.54	1.31	1.15	Hs.444354	NM_012164; F-box and WD-40 domain protein 2
202777_at	CIS	1.93	1.51	1.29	1.12	Hs.104315	NM_007373; soc-2 suppressor of clear homolog
200958_s_at	CIS	1.92	1.49	1.28	1.11	Hs.164067	NM_005625; syndecan binding protein (syntenin)
209579_s_at	CIS	1.79	1.36	1.16	1.01	Hs.35947	NM_003925; methyl-CpG binding domain protein 4
209004_s_at	CIS	1.63	1.21	1.00	0.89	Hs.5548	NM_012161; F-box and leucine-rich repeat protein 5 isoform 1 NM_033535; F-box and leucine-rich repeat protein 5 isoform 2
218150_at	CIS	1.6	1.18	0.98	0.86	Hs.342849	NM_012097; ADP-ribosylation factor-like 5 isoform 1 NM_177985;

							ADP-ribosylation factor-like 5 isoform 2
202076_at	CIS	1.53	1.12	0.92	0.82	Hs.289107	NM_001166; baculoviral IAP repeat-containing protein 2
204640_s_at	CIS	1.45	1.03	0.83	0.75	Hs.129951	NM_003563; speckle-type POZ protein
201887_at	CIS	1.32	0.92	0.74	0.66	Hs.285115	NM_001560; interleukin 13 receptor, alpha 1 precursor
212802_s_at	CIS	1.31	0.91	0.72	0.65	Hs.287266	
212899_at	CIS	1.29	0.89	0.71	0.64	Hs.129836	NM_015076; cyclin-dependent kinase (CDC2-like) 11

Feature: Probe-set on U133A GeneChip
Class: The group in which the marker is up-regulated
T-test: The t-test value
Perm 1%: The 1% permutation level
Perm 5%: The 5% permutation level
Perm 10%: The 10% permutation level

Exploration of strength of CIS classifier

To further explore the strength of classifying CIS we also built a classifier by randomly selecting half of the samples for training and used the other half for testing. Cross validation was used again in the training of this classifier for optimisation of the gene-set for classifying independent samples. Cross-validation with 15 genes showed a good performance (see figure 18) and 7 of these genes were included in 70% of the class-validation loops. These 7 genes classified the samples in the test set with one error only - sample 1482-1 (χ^2-test, $P<0.002$). Only two of the genes were also included in the 16-gene classifier, which is understandable considering the number of tests performed and the limitations in sample size. This classification performance is notable considering the small number of samples used for training the classifier.

Grouping of normal and cystectomies with CIS

We used hirarchichal cluster analysis to group the 9 normal and 10 biopsies from cystectomies with CIS based on the normalised expression profiles of the 16 classifier genes (Fig. 17b). This clustering separated the samples from cystectomies with CIS lesions from the normal samples with only few exceptions as 8 of the 10 biopsies from cystectomies were

found in the one main branch of the dendrogram and 8 of the 9 normal biopsies were found on the other main branch (χ^2-test, $P<0.002$).

Tables

Table B

References

Pisani, P., Parkin, D.M., Bray, F. & Ferlay, J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer* **83**, 18-29 (1999).

Wolf, H. et al. Bladder tumors. Treated natural history. *Prog Clin Biol Res* **221**, 223-55 (1986).

Althausen, A. F., Prout, G. R., Jr., and Daly, J. J. Non-invasive papillary carcinoma of the bladder associated with carcinoma in situ. J Urol, *116:* 575-580, 1976.

Spruck, C. H., 3rd, Ohneseit, P. F., Gonzalez-Zulueta, M., Esrig, D., Miyao, N., Tsai, Y. C., Lerner, S. P., Schmutte, C., Yang, A. S., Cote, R., and et al. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res, *54:* 784-788, 1994.

Rosin, M. P., Cairns, P., Epstein, J. I., Schoenberg, M. P., ånd Sidransky, D. Partial allelotype of carcinoma in situ of the human bladder. Cancer Res, *55:* 5213-5216, 1995.

Anderstrom, C., Johansson, S., and Nilsson, S. The significance of lamina propria invasion on the prognosis of patients with bladder tumors. J Urol, *124:* 23-26, 1980.

Cummings, K. B. Carcinoma of the bladder: predictors. Cancer, *45:* 1849-1855, 1980.

Cheng, L., Cheville, J. C., Neumann, R. M., Leibovich, B. C., Egan, K. S., Spotts, B. E., and Bostwick, D. G. Survival of patients with carcinoma in situ of the urinary bladder. Cancer, *85:* 2469-2474, 1999.

Kriegmair, M., Baumgartner, R., Lumper, W., Waidelich, R., and Hofstetter, A. Early clinical experience with 5-aminolevulinic acid for the photodynamic therapy of superficial bladder cancer. Br J Urol, *77:* 667-671, 1996.

van 't Veer, L.J. et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* **415**, 530-6 (2002).

Dyrskjot, L. et al. Identifying distinct classes of bladder carcinoma using microarrays. *Nat Genet* **33**, 90-6 (2003).

van Rhijn, B.W. et al. The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. *Cancer Res* **61**, 1265-8 (2001).

Yuan, J. et al. Polo-like kinase, a novel marker for cellular proliferation. *Am J Pathol* **150**, 1165-72 (1997).

Galaktionov, K. & Beach, D. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. *Cell* **67**, 1181-94 (1991).

Weinstein, J., Jacobsen, F.W., Hsu-Chen, J., Wu, T. & Baum, L.G. A novel mammalian protein, p55CDC, present in dividing cells is associated with protein kinase activity and has homology to the Saccharomyces cerevisiae cell division cycle proteins Cdc20 and Cdc4. *Mol Cell Biol* **14**, 3350-63 (1994).

Hiraiwa, A. et al. Immunolocalization of hCDC47 protein in normal and neoplastic human tissues and its relation to growth. *Int J Cancer* **74**, 180-4 (1997).

Liu, J. & Kern, J.A. Neuregulin-1 activates the JAK-STAT pathway and regulates lung epithelial cell proliferation. *Am J Respir Cell Mol Biol* **27**, 306-13 (2002).

Park, B.H., Vogelstein, B. & Kinzler, K.W. Genetic disruption of PPARdelta decreases the tumorigenicity of human colon cancer cells. *Proc Natl Acad Sci U S A* **98**, 2598-603 (2001).

Fauconnet, S. et al. Differential regulation of vascular endothelial growth factor expression by peroxisome proliferator-activated receptors in bladder cancer cells. *J Biol Chem* **277**, 23534-43 (2002).

Golub, T.R. et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* **286**, 531-7 (1999).

Ambrosini, G., Adida, C. & Altieri, D.C. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* **3**, 917-21 (1997).

Sidransky, D. et al. Clonal origin bladder cancer. *N Engl J Med* **326**, 737-40 (1992).

Primdahl, H. et al. Allelic imbalances in human bladder cancer: genome-wide detection with high-density single-nucleotide polymorphism arrays. *J Natl Cancer Inst* **94**, 216-23 (2002).

Eaves, I.A. et al. Combining mouse congenic strains and microarray gene expression analyses to study a complex trait: the NOD model of type 1 diabetes. *Genome Res* **12**, 232-43 (2002).

Ghandour, G. & Glynne, R. Method and apparatus for analysis of data from biomolecular arrays. *International patent # WO0079465* (2000).

Turkey, J. *Exploratory Data Analysis*, (Addison-Wesley, Reading, Mass, 1977).

Kruhoffer, M., Magnusson, N.E., Aaboe, M., Dyrskjot, L. & Orntoft, T.F. Microarrays for gene expression profiling: Fabrication of Oligonucleotide microarrays, Isolation of RNA, Fluorescent labelling of cRNA, Hybridisation, and Scanning. in *Cell Biology - A laboratory handbook* (ed. Celis, J.E.) (To appear in the 4th edition of this book).

Jain, A.N. et al. Fully automatic quantification of microarray image data. *Genome Res* **12**, 325-32 (2002).

Eisen, M.B., Spellman, P.T., Brown, P.O. & Botstein, D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* **95**, 14863-8 (1998).

Seymour, L. Novel anti-cancer agents in development: exciting prospects and new challenges. *Cancer Treat.Rev.* **25**, 301-312 (1999).

Fox, S. B., Gasparini, G., & Harris, A. L. Angiogenesis: pathological, prognostic, and growth-factor pathways and their link to trial design and anticancer drugs. *Lancet Oncol.* **2**, 278-289 (2001).

Shipp, M. A. *et al.* Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat.Med.* **8** , 68-74 (2002).

Kerr, J. S., Slee, A. M., & Mousa, S. A. Small molecule alpha(v) integrin antagonists: novel anticancer agents. *Expert.Opin.Investig.Drugs* **9**, 1271-1279 (2000).

Bergkvist, A., Ljungqvist, A., & Moberger, G. Classification of bladder tumours based on the cellular pattern. Preliminary report of a clinical-pathological study of 300 cases with a minimum follow-up of eight years. *Acta Chir.Scand.* **130**, 371-378 (1965).

Li, C. & Hung, W. W. Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. *Genome Biol.* **2**, RESEARCH0032 (2001).

Kononen, J. *et al.* Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat.Med.* **4**, 844-847 (1998).